JC10 Rec'd PCT/PTO 01 JUN 2001

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
PG3606USW

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
09/857123

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/09284	**30 November 1999**	**1 December 1998**

TITLE OF INVENTION
HUMAN VANILLOID RECEPTORS AND THEIR USES

APPLICANT(S) FOR DO/EO/US
DELANY, Natalie Samantha **TATE, Simon Nicholas**
SANSEAU, Philippe

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☒ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

   Copy of Request (PCT/RO/101)

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JC18 Rec'd PCT/PTO 01 JUN 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/857123	INTERNATIONAL APPLICATION NO. PCT/EP99/09284	ATTORNEY'S DOCKET NUMBER PG3606USW

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS	PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1000.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	51 - 20 =	31	x $18.00	**$558.00**	
Independent claims	5 - 3 =	2	x $80.00	**$160.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,578.00**	
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,578.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,578.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,578.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of ______________ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1392** in the amount of **$1,578.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347
PATENT TRADEMARK OFFICE

Frank Grassler
SIGNATURE

Frank Grassler
NAME

31,164
REGISTRATION NUMBER

June 1, 2001
DATE

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09/857123

JC18 Rec'd PCT/PTO 01 JUN 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of: DELANY et al.
International Application No.: PCT/EP99/09284
International Filing Date: 30 November 1999
Title: HUMAN VANILLOID RECEPTORS AND THEIR USES

Honorable Commissioner of Patents
Washington, D.C. 20231

FIRST PRELIMINARY AMENDMENT

Dear Sir:

The above-identified application is being transmitted herewith for entry in the US National Phase under Chapter II of the PCT for the purpose of adding the priority information. Please amend the application as follows:

In the Abstract:

The Abstract has been placed on a separate sheet of paper according to US practice, as required under 37 CFR 1.72(b).

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. §371 as a United States National Phase Application of International Application No. PCT/EP99/09284 filed 30 November 1999, which claims priority from Great Britain Application No. 9826359.3 filed 1 December 1998.--

In the Claims

14. (Amended) An expression vector comprising a nucleotide sequence according to claim 6, which is capable of expressing an hVR protein or a variant thereof.

23. (Amended) An antibody specific for a human vanilloid receptor (hVR) protein or a variant thereof as claimed in claim 1.

26. (Amended) A method for identification of a compound which exhibits hVR modulating activity comprising contacting a human vanilloid receptor (hVR) protein or a variant thereof according to claim 1 with a test compound and detecting modulating activity or inactivity.

45. (Amended) A method of producing an hVR protein or a variant thereof according to claim 1 comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant

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thereof, under conditions suitable for obtaining expression of the hVR protein or variant thereof.

50. (Amended) A human vanilloid receptor (hVR) protein according to claim 48, which is hVR1 or a variant thereof.

51. (Amended) A human vanilloid receptor (hVR) protein according to claim 48 which is hVR3 or a variant thereof.

REMARKS

Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information. Claims have been amended to eliminate multiple dependencies.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned **"Version with markings to show changes made."** Applicant respectfully requests the entry of the above preliminary amendments.

Examiner is invited and encouraged to contact the undersigned if such contact would facilitate prosecution of this application.

No fee is believed due in connection with this Amendment, however the Commissioner is hereby authorized to charge any under-payment to Deposit Account No. 07-1392.

Respectfully submitted,

Date: June 1, 2001

Frank P. Grassler
Attorney of Record, Reg. No. 31,164

GlaxoSmithKline
Corporate Intellectual Property Department
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709-3398
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CERTIFICATE OF EXPRESS MAILING (37 CFR 1.10)
I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Assistant Commissioner of Patents Washington, D.C. 20231 on 6/01/01

Marilyn Eldridge

Version with markings to show changes

14. An expression vector comprising a nucleotide sequence according to [any one of] claim[s] 6 [to 13].

23. An antibody specific for a human vanilloid receptor (hVR) protein or a variant thereof as claimed in [any one of] claim[s] 1 [to 5].

26. A method for identification of a compound with exhibits hVR modulating activity comprising contacting a human vanilloid receptor (hVR) protein or a variant thereof according to [any one of] claim[s] 1 [to 5] with a test compound and detecting modulating activity or inactivity.

45. A method of producing an hVR protein or a variant thereof according to [any one of] claim[s] 1[-5] comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVr protein or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or variant thereof.

50. A human vanilloid receptor (hVR) protein according to claim 48 [or 49] which is hVR1 or a variant thereof.

51. A human vanilloid receptor (hVR) protein according to claim 48 [or 49] which is hVR3 or a variant thereof.

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HUMAN VANILLOID RECEPTORS AND THEIR USES

Field of the Invention

The present invention relates to human vanilloid receptor (hVR) proteins and to related nucleotide sequences, expression vectors, cell lines, antibodies screening methods, compounds, methods of production and methods of treatment, as well as other related aspects.

Background of the Invention

Capsaicin, the irritant in hot peppers and a member of the vanilloid family activates a sub-group of sensory neurons: the nociceptors. These neurons transmit nociceptive and thermoceptive pain information back to pain-processing centres in the central nervous system such as the spinal cord and the brain. They are also sites for the release of pro-inflammatory mediators in the periphery (1). Nociceptors show heterogeneity in their sensitivity to capsaicin. Excitation and prolonged exposure of these neurons to capsaicin is followed by a refractory state known as desensitisation (2) when they become insensitive to capsaicin and other noxious stimuli (3). The long-term response to insensitivity could be explained by death of the nociceptors or destruction of its peripheral terminals (4). Because of the desensitisation phenomenon, capsaicin has been used therapeutically for decades as an analgesic agent for the treatment of pain in a range of disorders (5).

It has been speculated that the endogenous target for capsaicin plays an important function in the detection of painful stimuli. It has been shown by electrophysiological and biochemical studies that capsaicin induces a flux of cations in dorsal root ganglion (DRG) neurons (6,7). Because other vanilloid derivatives show responses in a dose dependent manner (8,9) a receptor is the most likely candidate to explain the mechanism. Therefore, based on indirect evidence it has been anticipated that these actions of capsaicin (excitation / desensitisation) are mediated by a specific membrane-bound receptor named vanilloid receptor (10).

Evidence for the existence of a vanilloid receptor came from binding experiments with resiniferatoxin (RTX), a capsaicin analog (11), and a competitive antagonist

of capsaicin, capsazepine (12). Vanilloid receptors have been visualised by using ($[^3H]$-RTX) autoradiography in dorsal root ganglia (DRG) and spinal cord of different species including man (13,14).

Recently, a rat vanilloid receptor termed VR1 has been identified using an expression-cloning strategy to isolate the complementary DNA (cDNA) encoding the corresponding protein from a rat DRG cDNA library (15). The cDNA clone was completely sequenced. The rat VR1 cDNA has an open reading frame of 2,514 nucleotides and encodes for a protein of 838 amino acids with a predicted relative molecular mass of 95,000. Analysis of the amino acid sequence identified 6 potential transmembrane regions with a short hydrophobic stretch between the transmembrane regions 5 and 6. The N-terminus (amino terminal) contains three ankyrin repeat domains. No motifs have been identified at the C-terminus (carboxy terminal).

It has been noted that rat VR1 transfected cells exhibit an increase in calcium levels after heat treatment and it has been suggested that *in vivo* VR1 and vanilloid receptors are involved in detection of noxious heat (but not innocuous heat). It has also been proposed that protons could act as modulators of the vanilloid receptors (16, 17, 18).

While it has been recognised that the rat capsaicin receptor, VR1, is a member of the family of non-selective ion channels that are gated by ligands and that it is involved in pain sensation, the natural ligand of VR1 remains unknown. It is therefore suggested that human vanilloid receptor sub-types may provide targets for the development of novel analgesic agents (agonists and antagonists) and agents which may interact with other disorders.

Accordingly, it is an object of the present invention to locate and characterise human vanilloid receptors. Other objects of the present invention will become apparent from the following detailed description thereof.

Summary of the Invention

According to one embodiment of the present invention there is provided an isolated human vanilloid receptor (hVR) protein or a variant thereof. Preferably

the hVR protein is an hVR1 or hVR3 protein or a variant thereof. In a particularly preferred aspect of the invention the hVR protein has an amino acid sequence as shown in figure 3 or in figure 18.

According to another aspect of the invention, there is provided a human vanilliod receptor (hVR) protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as diabetic neuropathy, incontinence and interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof as hereinbefore described, or a nucleotide sequence that is complementary thereto. Preferably the nucleotide sequence encodes an hVR1, hVR3 protein or variant thereof or a nucleotide sequence which is complementary thereto. Particularly preferably the nucleotide sequence is as shown in figure 2 and figure 17.

According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. Preferably the expression vector is as displayed in figure 6 or figure 20.

According to another aspect of the invention there is provided a stable cell line comprising an expression vector as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. The stable cell line is preferably a

modified mammalian cell line, preferably HEK293, CHO, COS, HeLa or BHK although transient expression may be preferred in *Xenopus* oocytes.

According to another aspect of the invention there is provided an antibody specific for an hVR protein as hereinbefore described or a variant thereof, preferably specific for hVR1 or hVR3 or a variant thereof.

According to another aspect of the invention there is provided a method for identification of a compound which exhibits hVR modulating activity, comprising contacting an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, with a test compound and detecting modulating activity or inactivity.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, for use in therapy.

According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR,

preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine, for use in therapy.

According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic

pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound identified by the method referred to above.

According to another aspect of the invention there is provided a compound identified by the method referred to above, for use in therapy.

According to another aspect of the invention there is provided the use of a compound identified by the method referred to above in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic

obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identified by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a method of producing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof.

Brief Description of the figures

Figure 1 is an alignment of hVR1 *in silico* derived clusters with rat VR1.

Figure 2 displays the human VR1 nucleotide sequence including the 5'UTR (nt –773 to nt 0), coding region (nt 1 to 2517) and 3'UTR (nt 2518 to nt 3560).

Figure 3 illustrates the nucleotide and encoded amino acid sequence of the human VR1sequence.

Figure 4 depicts the amino acid sequence of the hVR1 gene, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed). The predicted phosphorylation sites are underlined.

Figure 5 is a comparison of the amino acid sequences of the rat (rVR1) and human (hVR1) vanilloid receptors.

Figure 6 illustrates constructs pBluescriptSK(+) (A) and pCIN5-new (B) with the full length hVR1 gene cloned via NotI and EcoRI restriction sites.

Figure 7 shows a Slot Blot hybridisation with hVR1 probe with positive labelling of both rat and human DRG mRNA.

Figure 8 displays a Western blot probed with anti-VR1 antibodies with the arrow indicating the VR1 specific protein.

Figure 9 shows localisation of VR1 in rat DRG tissue sections, the arrow points to VR1 expressing small diameter (<25μn) neurone cell bodies.

Figure 10 depicts the *in situ* localisation of VR1 in human DRG sections (A) and human skin (B).

Figure 11 illustrates the functional response to capsaicin and blockade by capsazepine (CPZ) (A) with the current voltage relationship plotted in (B) on human VR-1 channels, transiently expressed in HEK293T cells.

Figure 12 shows capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium (A), maximum current (65mV) against time (B) and current voltage relationship in the absense of Ca^{2+} (C).

Figure 13 shows the influx of calcium into transiently transfected HEK293T cells over a time course in the presence of agonist capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 14 illustrates a graphical presentation the results shown in figure 13 examining the response of hVR1 transfected HEK293T cells over time before and after exposure to agonists: capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 15 displays the proposed assay strategy to carry out drug screening.

Figure 16 displays an alignment of *in silico* derived hVR3 specific clusters with rat VR1.

Figure 17 depicts the hVR3 nucleotide sequence including the 5' UTR (nt –686 to nt 0) Coding region (nt1 to nt 2889), 3'UTR (nt 2890 to nt 3418).

Figure 18 shows the nucleotide and amino acid sequence of hVR3.

Figure 19 is of the amino acid sequence of hVR3, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Figure 20 displays constructs pBluescriptSK(+) (A) and pCDNA3.1 (+) (B) with the full length hVR3 gene cloned via NotI and XhoI restriction sites.

Figure 21 illustrates a multiple comparison of the amino acid sequences of the rat VR1 and the human vanilloid receptors: hVR1, hVRL-1 and hVR3.

Figure 22 Northern Blot hybridisation with hVR3 probe with strong signals detected in trachea (A), prostate (B), placenta, kidney and pancreas (C).

Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

As referred to above, the present invention relates to isolated human vanilloid receptor (hVR) proteins, and in particular to the human vanilloid receptors which will be termed respectively human vanilloid receptors 1 and 3 (hVR1, and hVR3), sequence information for which is provided in figures 2 (hVR1) and 17 (hVR3). In the context of this invention the term "isolated" is intended to convey that the receptor protein is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the receptor protein being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the receptor protein is in a state as found in nature.

Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. *et al.* (28), the disclosure of which is included herein in its entirety by way of reference.

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the human vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example,

other peptides or proteins with greater than about 80%, preferably at least 90% and particularly preferably at least 95% homology with the sequences provided are considered as variants of the receptor proteins. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the biological functionality of a human vanilloid receptor. This biological functionality can of course be assessed by conducting binding studies with known vanilloid modulators such as capsaicin, capsazepine (12) and resiniferatoxin (11).

Human VR1 is preferentially expressed in human dorsal root ganglia (DRG) and relative to hVR3 has the highest sequence homology with the rat VR1. Therefore, hVR1 is likely to be the human orthologue to rat VR1. hVR3 is less similar to rat VR1 and is expressed in a wider range of tissues. Nucleotide sequence analysis of hVR1 reveals a 2517bp open reading frame which encodes an 839 amino acid protein (see figures 2, 3 and 4). This deduced hVR1 protein sequence is 86 % identical to the rat VR1 (15) and shares many of its characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains. Similarly hVR3 has an open reading frame of 2889bp open reading frame which encodes a 963 amino acid protein (see figures 17, 18 and 19). The deduced hVR3 protein is 46 % identical to rat VR1 and 44 % identical to hVR1 sharing many of VR1's characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains.

The invention also includes nucleotide sequences which encode for human vanilloid receptor proteins or variants thereof as well as nucleotide sequences which are complementary thereto. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. Preferably the proteins are hVR1, hVR3 or variants thereof. Such nucleotides can be isolated or synthesised according to methods well know in the art. See reference 28, the disclosure of which is included herein in its entirety by way of reference.

The present invention also includes expression vectors which comprise nucleotide sequences encoding for the hVR, preferably hVR1 or hVR3, receptor

proteins or variants thereof. A further aspect of the invention relates to an expression vector comprising nucleotide sequences encoding for hVR1 or hVR3 receptor proteins or variants thereof. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Suitable vectors for use in practicing the present invention include pBluescript (Stratagene), pCR-Script (Stratagene), pCR2.1-TOPO (Invitrogen), pCRII-TOPO (Invitrogen), pCR-Blunt (Invitrogen), with vectors such as pCIN (32) (available from Clontech as pIRES-neo), pCDNA 3.1 (Invitrogen) or pCIneo (Promega) required for mammalian expression. Appropriate methods can be effected by following protocols described in many standard laboratory manuals (28, 29).

The invention also includes cell lines which have been modified to express the novel receptor. Such cell lines include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which have been modified by insertion of vectors encoding for the receptor proteins according to the invention include HEK293T cells and *Xenopus* oocytes. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of the inventive receptor. Representive examples of appropriate hosts include animal cells such as HEK293, CHO, COS, HeLa and BHK.

It is also possible for the receptors of the invention to be transiently expressed in a cell line or on a membrane, such as for example in a baculovirus expression system. Such systems, which are adapted to express the receptors according to the invention, are also included within the scope of the present invention.

In particular, the functional hVR protein may include hVR receptor proteins selected from hVR1 and hVR3 and thereof or even other hVR protein subtypes or splice variants which have not yet been identified.

According to another aspect, the present invention also relates to antibodies, preferably monoclonal antibodies, which have been raised by standard techniques and are specific for the receptor proteins or variants thereof according to the invention. Such antibodies could for example be useful in purification, isolation or screening involving immuno precipitation techniques and may be used as tools to further ellucidate hVR, preferably hVR1 or hVR3, protein function, or indeed as therapeutic agents in their own right. Antibodies may also be raised against specific epitopes of the receptors according to the invention.

An important aspect of the present invention is the use of receptor proteins according to the invention in screening methods designed to identify compounds which act as receptor ligands and which may be useful to modulate receptor activity. In general terms, such screening methods will involve contacting the receptor protein concerned, preferably hVR1 or hVR3, with a test compound and then detecting modulation in the receptor activity, or indeed detecting receptor inactivity, which results. For further details on the screening strategy refer to figure 15. The present invention also includes within its scope those compounds which are identified as possessing useful hVR, preferably hVR1 or hVR3, modulation activity, by the screening methods referred to above. The screening methods comprehended by the invention are generally well known to persons skilled in the art. High throughput screens may include fluorescence based assays using the Fluorometric Imaging Plate Reader (FLIPR) with calcium sensitive dyes, and reporter gene assays using calcium sensitive photoproteins that emit light on the influx of calcium and can be detected using an Imaging system. Secondary screens may involve electrophysiological assays utilising patch clamp technology to identify small molecules, antibodies, peptides, proteins or other types of compounds that interact with hVR, preferably hVR1 or hVR3, to modulate activity. Tertiary screens may involve the study of modulators in well characterised rat and mouse models of pain. These models of pain include, but are not restricted to, intraplantar injection of inflammatory agents such as carageenan, formalin and complete freunds adjuvant (CFA). Models of neuropathic pain such as loose ligature of the sciatic nerve are also included. Other screens may involve the study of modulators in human volunteers subject to topically applied capsaicin.

Another aspect of the present invention is the use of compounds which have been identified by screening techniques referred to above in the treatment or prophylaxis of disorders which are responsive to modulation of hVR, preferably hVR1 or hVR3, receptor activity, in a human patient. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine. hVR , preferably hVR1 and hVR3, proteins have been implicated in disorders of the central nervous system (CNS), gastrointestinal (GI) tract, lungs and bladder and therefore modulation of hVR, preferably hVR1 or hVR3, receptor activity in these tissues will result in a positive therapeutic outcome in relation to such disorders. In particular, the compounds which will be identified using the screening techniques according to the invention will have utility for treatment and/or prophylaxis of disorders such as pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, IBS, respiratory disorders such as asthma and COPD, urological disorders including diabetic neuropathy, incontinence and interstitial cystitis, and inflammatory disorders. It is to be understood however, that the mention of such disorders is by way of example only, and is not intended to be limiting on the scope of the invention.

The compounds which are identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remmington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference.

The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

The present invention will be further explained, by way of examples, in the appended experimental section. Reference examples are provided.

Experimental details

Reference Example A: Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by *in silico* analysis

The full-length rat VR1 amino acid sequence (15) was used as a query sequence using the tBlastn (20) alignment program to identify related human genes in the dbEST (21) and Incyte (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-acid identity and 84% similarity over a region of 70 amino acids (15). Full-length cloning and functional characterisation of the gene represented by this cluster has been completed (30). This gene was denoted hVRL-1 and encoded a protein of 764 amino acid protein that was 48 % identical to the rat VR1 protein. All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered into ten groups, one of these clusters represented hVRL-1. The remaining nine clusters have been named hVRa, hVRb, hVRc, hVRd, hVRe, hVRf, hVRg, hVRh and hVRi. For each EST the tissue source was assigned according to the annotations in the dbEST and Incyte databases. Since no obvious starting codon was present and the cluster sequences were shorter than the rat VR1 transcript none of these clusters were likely to represent a full-length vanilloid receptor transcript. Finally hVRg, hVRh and hVRi collapsed into a single contig. Sequence analysis has shown that

these cDNAs are likely to be chimeric. The 5' end has weak similarities with the rat VR1 gene but the 3' end is identical to a DNA binding protein. No more work was pursued with that transcript.

Reference Example B: Isolation of the human orthologue to the rat VR1 gene (reference examples B1-B4):

Reference Example B1: ***In silico*** **assembly of human VR1**

The consensus nucleotide sequences from the ten clusters were searched with the tBlastx program (20) against the rat VR1 sequences to identify the most likely open reading frames. Frame shifts were corrected when the sequence trace files were available. Each cluster was aligned against the rat VR1 amino-acid sequence according to the Blastx results. The Blastx alignment program (20) was used to compare the full-length rat VR1 protein with the amino-acid sequences of the ten clusters. The three clusters with the highest homology, displayed in figure 1, were aligned with the rat VR1 gene.

Cluster hVRa shared a high homology (70% identity and 75% similarity over a stretch of 107 amino acids) with the 5' of the rat VR1 sequence but did not seem to have a potential start codon. It contained two ESTs (EST1 and EST2) derived from the same tissue, bladder, and from the same patient. These two ESTs were selected for further investigation since this cluster was the most 5', had high homology with rat VR1 and the bladder tissue could be contaminated with sensory neurones. Both cDNA clones were ordered but only clone EST1 was received as EST2 failed the recovery procedure.

Cluster hVRb composed of two EST's (EST3 and EST4), with 89% identity and 92% similarity over 90 residues, showed the highest degree of homology to the rodent sequence. The overlap between both sequences was located towards the middle of the gene.

hVRc (EST5) also while having high homology (71% identity and 75% similarity over 65 residues) with rat VR1 was closely related to the C-terminus of the rat protein sequence.

Reference Example B2: Sequencing of clones

All DNA sequences were determined by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A / 377 sequencers (Applied Biosystems). Sequence-specific primers were used with the 'Big-Dye' Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analysed using programs from the University of Wisconsin Genetics Computer Group package.

More specifically when sequencing an EST clone, the following protocol was adopted. The EST1 clone was grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) and T7 (5' TAATACGACTCACTATAGGG) primers flanking the cloning site were used to sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to EST1). The T7 end did not have homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif. The size of the insert was determined by enzyme digestion of the DNA with the endonucleases NotI and EcoRI and calculated to be approximately 3kb.

Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 minute and terminated by 5 minutes at 72°C. The resulting PCR amplicon was separated on a 1.2% agarose gel and shown to be of ~3kb in size.

To fully sequence the PCR product the nuclease-Bal-31 technique was used where both strands of duplex DNA are degraded from both ends (23). After ethanol precipitation of the PCR product, the pellet was re-suspended in 30ml of 1X Bal-31 buffer (add buffer composition). A time-course digest with 2 units of Bal-31 enzyme (Roche Molecular Biochemicals) was carried out with 12 time points taken over 90 minutes (30 seconds, 1, 2, 3, 5, 7, 10, 15, 25, 45, 75 and 90 minutes). Three pools were made respectively from digests 1 to 4, 5 to 8 and 9

to 12. Each pool was blunt-ended and sub-cloned into the pCR-Script SK (+) plasmid from Stratagene at the SrfI site. After transformation, 16 colonies from each pool were screened by PCR with the flanking Reverse (5' GGAAACAGCTATGACCATG) and M13-20 (5' GTAAAACGACGGCCAGT) primers. The amplicons of 6 positive colonies per pool were subjected to direct sequencing (24) using the T3 (5' AATTAACCCTCACTAAAGGG) and T7 primers. The DNA sequences obtained were assembled using the GCG package, translated and aligned against the rat VR1 gene using the Blast tools. After analysis, the 3079bp amplicon was shown to have 2 introns of 603bp and 1221bp. The latter intron was located at the 3'end of the PCR product. The coding sequence covered 1255 bp and was separated by the former intron. Therefore the clone EST1 was likely to be a partially spliced and incomplete cDNA.

The clone belonging to cluster 1b (EST3) and derived from a kidney cDNA library was ordered and sequenced using the Bal-31 technique. After assembly of the sequences using the GCG package an identical overlap was identified with the DNA sequence of the cluster hVRc. Moreover a 3'end with a polyadenlyation signal and tail was identified. The complete sequence of the combined hVRb Bal-31 derived sequence and hVRc was 2063 bp (1020 bp of coding and 1043 bp of 3' untranslated sequence).

Reference Example B3: Amplification of the middle section of hVR1 using the Polymerase Chain Reaction

We formulated the hypothesis that both sequences (hVRa and hVRb/c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from brain tissues in order to clone the gap. A smear was obtained with the sense primer (5' TCTACTTCGGTGAACTGCCC) and antisense (5' ACGGCAGGGAGTCATTCTTC). For specificity 50ng of the PCR product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) and antisense (5' GTCACCACCGCTGTGGAAAA) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVRa at one end and

hVRb/c at the other end. The middle part of the PCR product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVRa, hVRb/hVRc and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 824 amino acids followed by a 3' untranslated sequence of 1043 bp. The human amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence.

Reference Example B4: Isolation of the 5' Terminus of hVR1 by PAC isolation

Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) and antisense (5' GAACTGGGCAGAAAGTGCCT) were designed to amplify a 150bp product from the first intron mentioned in reference example B2. A P1 Artificial Chromosome (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) to sequence the genomic clone towards the potential 5' end of the gene. An open reading frame with a starting codon was identified. The gene structure was confirmed by using the GenScan software (27). The complete gene has a nucleotide sequence of 2517bp (figure 2) and encoded a 839 amino acid protein (Figures 3 and 4). The gene was named hVR1. Multiple alignment of the amino acid sequence of hVR1 and rat VR1 shows a remarkable degree of identity and similarities between both sequences (figure 5). The rVR1 and hVR1 amino acid sequences are 86% identical. Moreover after protein analysis 6 trans-membrane domains and 3 ankyrin binding domains were identified in hVR1 as in the rat VR1 gene.

Example 1: Full-length Amplification of hVR1 from human DRG and assembly into cloning vectors

HVR1 was PCR amplified in three sections from human DRG template. The 5' fragment was amplified using a sense primer encoding a NotI site and a strong

Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCGCCACCATGAAGAAATGGAGCAGCAC) and an antisense primer (5' AGGCCCACTCGGTGAACTTC). The thermo-cycling conditions used for this amplification included a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 5 min completed the reaction. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle section of hVR1 was PCR amplified using the sense primer: 5' GACGAGCATGTACAATGAGA and antisense primer: 5' GTCACCACCGCTGTGGAAAA. The cycling conditions included a hot start at 94°C for 4 mins, followed by 35 cycles of 1 min at 94°C, 56°C and 72°C. A final extension step of 72°C for 5 min completed the reaction. A band of approximately 870 bp was excised from a 2 % agarose gel and cloned as detailed by the TOPO™ TA Cloning® kit into the vector pCR2.1®-TOPO. Finally the 3' end was PCR amplified with the sense primer: 5' TGTGGACAGCTACAGTGAGA and the antisense primer: 5'TGCACTGAATTCGAGCACTGGTGTTCCCTCAG which encoded an EcoRI site for cloning. The PCR conditions included a 90 sec hot start at 94°C followed by 35 cycles of 94°C for 50 sec, 50°C for 50 sec and 72°C for 50 sec. The cycling was completed with a 72°C step for 5 min. PCR products were separated on a 2% agarose gel and cloned into the vector pCR2.1®-TOPO.

Resulting clones for each of the three hVR1-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full length assembly of the gene. The NotI/DraIII (New England Biolabs) digested 5' end fragment ligated together with the middle DraIII/EcoRI fragment into a NotI/EcoRI restricted pBluescript SK (+) vector (Stratagene). Finally, the remaining 3' fragment was introduced into the resulting construct via MscI and EcoRI restriction sites, a map of the resulting construct is displayed in figure 6A.

Several clones were selected for sequence analysis to confirm that constructs still encoded the hVR1 consensus sequence. These were then digested with NotI/EcoRI and ligated into the mammalian expression vector pCIN5-new (a modified version of pCIN1 (32) having an IVS deletion as well as a 36 bp

deletion repositioning the start codon of neomycin phosphotransferase immediately after the upstream EMVC IRES) as illustrated in figure 6B.

Example 2: Chromosomal Localisation

The primers used to isolate the PAC clone (reference example B4) were selected for PCR on the G3 radiation hybrid panel from Stanford commercially available from Research Genetics (Huntsville, Alabama). The positive lanes and negative patterns were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR1 gene appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

Example 3: mRNA Distribution

The tissue distribution of hVR1 was established by slot-blot hybridisation. RNA was transferred onto a sheet of GeneScreen hybridisation transfer membrane (DUPONT) sandwiched in a slot blotter by suction via a vacuum pump. Once the membrane was rinsed in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) for 2 min it was exposed to UV using an Ultraviolet crosslinker (Amersham Life Science) for 1min at 15000uW/cm^2 thus enabling cross-linkage of the RNA onto the membrane. The amounts of RNA on the blot are unknown. The probe was obtained by PCR amplification of a 260 bp product of the coding region of hVR1 with the following two primers: 5' TGTGGACAGCTACAGTGAGA and 5' GTGGAAAACCCGAACAAGA. Membranes were hybridised for 4 hr shaking at 60°C in a 10% dextran sulphate, 1% SDS (sodium dodecyl sulphate) and 1M NaCl solution. The probe was labelled with [α32P]dCTP (Amersham) using the Rediprime™DNA labelling system (Amersham), so as to obtain approximately 500,000cpm of the labelled probe per ml of prehybridisation solution. Briefly 100ng of probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45µl. This was added to the labelling tube from the kit together with 3µl of 32P dCTP followed by an incubation at 37°C for 30 minutes. 400µl of Herring Sperm DNA (Sigma) at a concentration of 8µg/ml was added to the labelled probe and heated at 99°C for 3 minutes followed by rapid cooling on ice. The labelled probe was added and mixed well in pre-hybridisation solution. The membranes were hybridised overnight at 55°C.

The membranes were then washed, first at room temperature in 2xSSC and 1% SDS for 5 minutes, followed by 2x SSC and 1% SDS for 30 min at 50°C. If necessary further washes with 1x SSC and 0.5% SDS or 0.1xSSC and 0.1% for 30 mins at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at –70°C overnight and the film developed.

The results are shown on figure 7. Strong signals were observed with the positive controls (slots 4B and 5B). Signals are detected on the human DRG slots (1A and 1B). No signals were detected with the water control (slot 3B). Three multi-tissue northern blots (Clontech) with a wide range of tissues have also been hybridised with the same probe, however no signals were detected. RT-PCR was performed on various tissues with the primer combination used to amplify the probe. A strong band was detected in DRG RNA. Taken together these hybridisations suggest that hVR1 is specifically expressed in neuronal tissue and DRG in particular.

Example 4: Design and production of Anti-hVR1 Antibody

The peptides CHIFTTRSRTRLFGKGDSEEASC (peptide68) and CGSLKPEDAEVFKDSMVPGEK (peptide69) were synthesised by standard solid phase techniques and purified by gel filtration chromatography. These peptides were conjugated via their Cys residues to the carrier protein, Tuberculin PPD (purified protein derivative) using sulpho-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexan-1-carboxylate). Rabbits, previously sensitised to Bacillus Calmette Guerin (BCG), were inoculated with the resulting conjugates emulsified in incomplete Freund's adjuvant at approx monthly intervals. Serum was prepared from blood samples taken 7 days after each immunisation. The specific antibody response was followed by indirect enzyme-linked immunosorbent assay (ELISA) using free peptide as antigen. Immunoglobulins were purified from high titre sera using immobilsed peptide affinity columns (sulpholink Pierce). Rabbits designated M143, 144 and 145 received peptide68 conjugate, rabbits M146, 147 and 148, peptide69 conjugate.

The antibodies have been validated by specific staining of the recombinant protein expressed in HEK293 cells. Whole cell lysates were prepared in Sample

Buffer (4 ml dH_2O, 1 ml 0.5 M Tris-HCl, pH 6.8, 0.8 ml glycerol, 1.6 ml 10 % w/v SDS, 0.4 ml 2-β mercaptoethanol and 0.2 ml of 0.05 % w/v bromophenol blue) and proteins separated by SDS-PAGE and transferred to a nitrocellulose filter by electroblotting. Following incubation with the antisera, bound immunoglobulins were revealed using HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL) detection. The antisera showed specific binding to a protein(s) of the appropriate molecular weight(s) in extracts of VR1 transfected cells, but not in control extracts, this is illustrated in figure 8.

Example 5: *Insitu* localisation of hVR1 using specific antibody

The purified immunoglobulins have been used for immunohistochemical staining of rat DRG tissue sections. Fixed cryosections of DRG were incubated with antibodies for 48h at 4°C at concentrations between 0.1 to 0.5μg/ml. Following a washing step, bound antibodies were detected by indirect immunofluorescence. The antibodies recognised exclusively small diameter cell bodies of the peripheral sensory neurones as displayed in figure 9. This observation has been extended to human DRG tissues for the anti-peptide68 peptide antibodies demonstrating cross-reactivity with the human sequence as expected. Figure 10A demonstrates labelling of DRG cell bodies with an arrow that points to small diameter neuronal cell body) and in figure 10B the arrow points to labelled neurones innervating human skin.

Example 6: Mammalian Cell Expression (examples 6a-6b)

Example 6a: Transient expression of hVR1 in mammalian cells

HEK293 cells were plated onto a 6 well plate, containing poly-l-lysine coated coverslips, at 5 x 10^4 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 8ug hVR1pCIN5, 2μg pEYFP-N1 reporter DNA, 12.4 μl calcium solution and water to 100μl. Solution B (hepes buffered saline) was slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture

medium was added and the plate was incubated 24-48 hours for functional analysis.

Example 6b: Stable expression of hVR1 in mammalian cells

HEK293 cells were plated onto a 6 well plate at 1 x 10^5 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 2μg hVR1pCIN5, 12.4μl 2M calcium solution and water to 100μl. Solution B (hepes buffered saline) was slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture medium was added and the plate was incubated 48 hours at 37°C, 5% CO_2. Cells were harvested into 100mm dishes in selection medium containing 800μg/ml geneticin. Cells were then incubated and fed at 4 day intervals. In total around 10 days selection is required for each single cell to multiply into a visible clone. Well-separated clones were each picked (with a gilson tip) into separate wells of a 96 well plate, containing maintenance medium (400μg/ml geneticin). Cells were expanded into flasks for freezing stocks and functional analysis. Stable cells may be plated at 1 x 10^5 cells onto poly-l-lysine coated coverslips in 6 well plate, for calcium imaging next day.

Example 7: Functional Analysis of hVR1(examples 7a-7c):

Example 7a: Electrophysiology using patch clamp methods

The activation of human VR-1 channels transiently expressed in HEK293T cells by capsaicin was investigated. Cells grown on poly-L-lysine-coated glass coverslips were placed in a recording chamber (0.5ml) and superfused with extracellular solution (2ml min^{-1}). The extracellular solution contained: NaCl (140mM), KCl (5mM), MgCl2 (2mM), CaCl2 (2mM), 4-(2-hydroxethyl)-1-piperazineethanesulphonic acid (HEPES, 10mM) and glucose (10mM). The pH was adjusted to 7.4 with NaOH and osmolarity ranged from 310-320mOsm l^{-1}. Patch pipettes (borosilicate glass) were pulled using a Sutter P-97 electrode puller. The pipettes were filled with an internal solution consisting of: CsCl

(140mM), ethylene glycol-bis(β-aminoethyl ether) *N,N,N',N'*-tetra acetic acid Cs salt (Cs-EGTA, 5mM) and HEPES (10mM). The pH was adjusted to 7.25 using CsOH and the osmolarity ranged from 275-290 mOsm. When filled with this internal solution, patch electrodes had resistances of 2-5 MΩ. Currents were recorded using standard whole-cell voltage clamp recording techniques (31) at room temperature (21-23°C) using an Axopatch 200A amplifier and signals were sampled at 2 or 0.1 kHz. The majority of series resistance errors (80-85%) were minimized with compensation circuitry. Membrane potentials were not corrected for junction potentials (<4 mV). Voltage pulses and data collection were performed on-line using pClamp8 software (Axon Instruments) interfaced with amplifiers. Membrane potentials were maintained at –60mV between protocols.

Capsaicin or capsazepine (CPZ) were applied, using a 'fast-flow sytem', directly onto the recording cell (<1s to equilibrate). The effects of capsaicin were measured either by application during constant recording while holding the membrane potential at –60mV to elicit an inward current, or applying voltage ramps (-100 to +60mV) in the absence and presence of capsaicin. Similarly both these methods of recording currents evoked by the application of capsaicin were used to demonstrate the blockade by the antagonist (CPZ).

Figure 11A reveals that application of capsaicin (1 μM), on human VR1 channels transiently expressed in HEK293T cells, produces an inward current when the membrane was held at a potential of -60mV. This response was abolished by 1μM CPZ and the blockade was partially reversible.

In the presence of 1 μM capsaicin, voltage ramps (-100 to +70mV) produced a current-voltage relationship demonstrating a substantial outward rectification. Addition of 1μM CPZ completely blocked the current (figure 11B). Again, only partial recovery was observed, especially for the inward currents evoked by negative potentials.

Capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium is illustrated in figure 12. Voltage ramps (-100 to +70) were applied and the addition of capsaicin (1μM) evoked an outwardly rectifying current. Repeated additions of capsaicin resulted in a progressive 'rundown' in

the size of the response (figure 12A). Figure 12B shows a plot of the current elicited at a potential of +65mV against time illustrating the 'rundown' in current amplitude. Voltage ramps were applied every 20s and capsaicin added at 2min intervals for approximately 40s. By the 6th addition the current had reduced about 4-fold.

When the external calcium was replaced with 5mM EGTA the size of the current increased dramatically (figure 12C). However, when calcium was re-applied to the external solution, the current evoked by capsaicin (1μM) was approximately equivalent to that of the 6th addition shown in (figure 12A).

Example 7b: Calcium Imaging with HEK293 expressing hVR1

HEK293 cells expressing hVR1 transiently or stably, were plated onto poly-l-lysine coated cover slips at 1 x 10^5 cells per well. They were analysed on the following day by calcium imaging (QuantiCell 700, Applied Imaging). On the day of experiment, WASH buffer was prepared by adding $CaCl_2$ to extracellular medium (ECM) to a final concentration of 2mM, (ECM contains 125mM NaCl, 5mM KCl, 2mM $MgCl_2$, 0.5mM NaH_2PO_4, 5mM $NaHCO_3$, 10mM Hepes, 10mM glucose, 0.1% BSA, pH7.4). The calcium sensitive dye solution was prepared by adding 50μl 5% pluronic F-127 in DMSO (Molecular Probes) to a vial of fura2-AM (Molecular Probes). After mixing, 20μl of the fura2-AM solution was added to 10ml WASH. 1.5 ml was then added to cells, which were then incubated at 37°C for 30 minutes. The plate was washed three times with WASH. 1ml WASH was added and stored in dark. Agonists and antagonists were prepared in WASH at 5x their required assay concentrations. The reagents and assay temperature was kept at 37°C. For the transiently transfected cells, the YFP reporter DNA fluorescence (490nm excitation) was used to identify the transfected cells. Cells were initially imaged in 400μl WASH (or 300μl WASH plus 100μl antagonist e.g. capsazepine). After approximately 1 min, 100μl agonist (e.g. capsaicin, anadamide or resiniferatoxin) at 5 x the desired concentration was added to give final 1x concentration. A sequence of images (340/380nm excitation) were taken to monitor calcium influx response in cells before (30-60 secs), and after the addition of agonist (2-5 mins). Figure 13 displays time courses taken for each of the tests set up to look at the affect of the different agonists mentioned above in the presence or absence of the rat VR1 antagonist, capsazepine. The Imager

also plots graphs of respective calcium concentration (nM) versus time (seconds) as shown in figure 14. After the addition of agonist (e.g. capsaicin, indicated by the vertical arrow on graph), the cells expressing hVR1 are stimulated to influx calcium. This is shown by the appearance of peak on the trace. The peak height correlates with hVR1 expression level. Varying levels of expression is some times seen depending on which cells are selected for the graph. Similar experiments may be accomplished to examine the response of protons and heat.

Example 7c: Use of a FLIPR assay with VR1

FLIPR (Fluorometric Imaging Plate Reader) is a high throughput fluorescence-based drug discovery tool for functional cell analysis. Intracellular calcium is monitored with the calcium sensitive dye, fluo3-AM. HEK293 cells stably expressing rat VR1 were plated into a 96 well, poly-l-lysine treated FLIPR plate at 3 x 10^4 cells per well. On the following day, the plate was processed for FLIPR. FBP buffer was prepared (15µM Probenecid (calcium ATPase pump blocker) in 1x FLIPR buffer (145mM NaCl, 5mM KCl, 1mM MgCl2, 2mM $CaCl_2$, 10mM glucose, 20mM Hepes). FBP buffer pH was then adjusted to 7.4 with NaOH. 400µl DMSO was added to a vial of fluo3-AM (Cambridge Bioscience, F-1241). The fluo3-AM solution was incubated at 37°C for 10 min and vortexed. LOAD was prepared by adding 20µl of fluo3-AM solution and 20µl 20% pleuronic F-127 in DMSO (Cambridge Bioscience, P-3000) into 10 ml FBP. The 96 well plate containing cells was flicked off to remove cell medium. 100µl LOAD was added per well. Cells were then incubated at 37°C for 60 minutes. Capsaicin (a rVR1 agonist) and capsazepine (CPZ, a rVR1 antagonist) were prepared at 10x the desired final assay concentrations in FBP. The plate was flicked to remove LOAD from cells, and 180µl FBP was added per well. The FLIPR machine added 20µl capsaicin per well to give a final 1x concentration. Cells were monitored for 70 seconds after agonist addition. The FLIPR traces (fluorescence change (counts) versus time (seconds)) were produced for each well. Peaks indicate capsaicin-gated calcium influx, by cells expressing rVR1. The peak height correlates with the rVR1 expression level. To measure antagonism of the VR1 response 20µl 10x antagonist CPZ was added into wells to give a final 1x concentration. The plate was incubated for 15 minutes at room temperature prior reading in the FLIPR. The FLIPR traces recorded for each well show that the

peak heights are reduced in cells pre-incubated in CPZ. The same FLIPR assay may be used to monitor the response of human VR1 on exposure to agonists and antagonists.

Example 8: Example of a screen using human VR1.

FLIPR assay technology may be utilised to screen for hVR1 modulators according to the procedure described in figure 15. Human VR1 may be gated with protons, capsaicin or heat.

Reference Example C: Identification and partial characterisation of additional human vanilloid receptors (reference examples C1-C3):

Reference Example C1: Identification and characterisation of a novel vanilloid–like receptor, hVR3

ESTs belonging to the remaining clusters were characterised by *in silico* cloning (reference example A). The following clones were used during this process: -EST6/EST7 (hVRd), -EST8. (hVRe), - EST9/EST10. (hVRf). These EST clusters have been aligned with rat VR1 in figure 16, note that this diagram is not to scale.

Reference Example C2: Sequencing of clones

Further sequencing, as detailed in reference example B2, and *in silico* cloning, enabled clusters hVRd, hVRe and hVRf to collapse forming a single contig of 583 amino acids. This sequence was named hVR3 and has 49 % identity with the rat VR1 sequence. It was unlikely that this single contig was a full-length vanilloid receptor transcript as no obvious starting codon was present and it was shorter than the rat VR1 transcript.

Reference Example C3: Identification of the 5' terminus of hVR3

Two primers (sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG) designed to PCR amplify an amplicon stretching the 3' end of hVR3 and its 3'utr were used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri). The hVR3 specific PAC clone was then used as template to generate a library. This was achieved by sonicating 6μg of Qiagen purified PAC construct, size selecting fragmented DNA of 500-

2000bp. These resulting fragments were then blunt ended and cloned into the vector pCR®-Blunt as detailed in the manufacturers protocol supplied with the Zero Blunt™ PCR cloning kit (Invitrogen). Clones were then sequenced (reference example B2) to identify the complete 5' end of the hVR3 transcript. The full-length nucleotide sequence of the hVR3 gene is displayed in figure 17. Figure 18 illustrates both nucleotide and encoded amino acid sequence of the human VR1 and figure 19 depicts the amino acid sequence of the hVR3 gene with shaded regions denoting predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Example 9: Full-length Amplification of hVR3 from human kidney template

Human kidney was used as a source of template for the PCR amplification of hVR3. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5' end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCACCATGCCCAGGGTAGTTGGAC and antisense primer (5' CACCTCTTGTTGTCACTGGA). The PCR conditions used were a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and finally one cycle at 72°C for 5 min. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle fragment was PCR generated using sense and antisense primers 5' CAAATCTGCGCATGAAGTTCCAG and 5' GCCACGAGAAGTTCCACGTAGTG respectively in the presence of 5% DMSO. PCR thermo-cycling required 35 cycles of 1 min at 94°C, 58°C and 72°C for successful amplification of the fragment which was then excised from a 2% agarose gel for cloning into the pCRII®-TOPO vector. Finally the 3' fragment was amplified with a sense primer 5' GCTGCTCCCATTCTTGCTGA and an antisense primer 5' TGCACTCTCGAGAAATGAGTGGGCAGAGAAGC encoding a XhoI restriction site. This fragment was successfully amplified using a hot start at 94°C for 4 min followed by 35 cycles of 94°C for 50 sec, 48°C for 50 sec and 72°C for 2 min. The cycling was completed with a 72°C step for 5 min. The amplified fragment was excised from a 2% agarose gel and clone into the pCRII®-TOPO vector.

Resulting clones for each of the three PCR generated hVR3-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full-length assembly of the gene. The DraIII restriction site of the pBluescript SK (+) vector (Stratagene) was firstly abolished by digestion with DraIII followed by a blunt ending step using T_4 DNA polymerase (New England Biolabs). This modified vector was then restricted to enable the ligation of both a NotI/NcoI 5' fragment and NcoI/ EcoRI middle fragment. Finally, the remaining 3' fragment was introduced into the resulting construct via DraIII and XhoI sites (figure 20A).

Several clones were selected for sequence analysis to confirm that the constructs still encoded the hVR3 consensus sequence. These were then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1 (+) (Invitrogen) as seen in figure 20B. The resulting hVR3 consensus sequence is shown in the multiple alignment along with the full-length sequence of hVR1 and the published hVRL-1 in figure 21.

Example 10: Chromosomal localisation

The 3' terminus, including the 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG. The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive and negative lanes were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

Example 11: mRNA distribution

The following primers (5' ACAAGAAGGCGGACATGCGG and 5' ATCTCGTGGCGGTTCTCAAT) were used to obtain a PCR product from the coding region of hVR3. This amplicon was used as a probe on multi-tissue northern blots, the protocol of which is detailed in example 3, to determine the tissue distribution of the gene (figures 22A, 22B and 22C). A transcript of approximately 3.8 kb was detected in the following tissues (the intensities of the

signals are indicated in brackets): trachea (very strong), kidney (strong), pancreas (strong), prostate (strong), placenta (strong), bone marrow (weak), adrenal gland (weak), lymph node (weak), spinal cord (weak), thyroid (weak), stomach (weak), lung (weak) and liver (weak).

Since these commercial blots (Clontech, Palo Alto, California, USA) should have the same amount of RNA it is interesting to note the very strong signal in the trachea lane (figure 22A). This could indicate the potential of hVR3 as a target for respiratory pathologies. It was shown by RT-PCR with the primer combination used to produce the probe that the gene is not expressed in DRG.

Example 12: Riboprobe generation for the in situ localisation of hVR3

The same probe, which was specific to hVR3 in Northern blot analysis (example 11), was used to generate a riboprobe. This hVR3 specific probe was cloned into the T7 and SP6 encoding pCRII®-TOPO vector (Invitrogen). This construct was then used in the *in vitro* transcription of DIG labelled RNA strands from the vectors promoters as described in the manufacturers instructions as detailed in the DIG RNA labelling kit (Roche Molecular Biochemicals). This riboprobe may be used to identify the cellular localisation of hVR3 present in tissues such as trachea, lung, pancreas, prostate, placenta and kidney.

Example 13: Mammalian Cell Expression of hVR3

Expression of hVR3 may be accomplished by transfecting a mammalian cell line such as: HEK283T, HEK293, CHO, COS, HeLa and BHK. A detailed method for both transient and stable transfection is detailed in example 6.

Example 14: Functional Analysis of hVR3

The functional analysis of hVR3 may be studied using the electrophysiology, calcium imaging and FLIPR methods as detailed in examples 7a to 7c.

Example 15: Example of a drug screen using human VR3.

A stable cell line expressing hVR3 may be used in a drug screen such as a selectivity screen using test compounds that have been identified to have an agonistic or antagonistic action on hVR1. FLIPR assay technology may be utilised to screen for hVR3 modulators as proposed in figure 15.

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 147 ccg acg ctg aag ctg gag gag ctc acc aac aag aag gga atg acg ccg    1785
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                    PATENT APPLICATION:  US/09/857,123   TIME: 10:54:46

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148 Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr Pro
149             325                 330                 335
151 ctg gct ctg gca gct ggg acc ggg aag atc ggg gtc ttg gcc tat att    1833
152 Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr Ile
153         340                 345                 350
157 ctc cag cgg gag atc cag gag ccc gag tgc agg cac ctg tcc agg aag    1881
158 Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg Lys
159     355                 360                 365
161 ttc acc gag tgg gcc tac ggg ccc gtg cac tcc tcg ctg tac gac ctg    1929
162 Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp Leu
163 370                 375                 380                 385
165 tcc tgc atc gac acc tgc gag aag aac tcg gtg ctg gag gtg atc gcc    1977
166 Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile Ala
167                 390                 395                 400
169 tac agc agc agc gag acc cct aat cgc cac gac atg ctc ttg gtg gag    2025
170 Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
171             405                 410                 415
173 ccg ctg aac cga ctc ctg cag gac aag tgg gac aga ttc gtc aag cgc    2073
174 Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
175         420                 425                 430
177 atc ttc tac ttc aac ttc ctg gtc tac tgc ctg tac atg atc atc ttc    2121
178 Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile Phe
179     435                 440                 445
181 acc atg gct gcc tac tac agg ccc gtg gat ggc ttg cct ccc ttt aag    2169
182 Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe Lys
183 450                 455                 460                 465
185 atg gaa aaa att gga gac tat ttc cga gtt act gga gag atc ctg tct    2217
186 Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu Ser
187                 470                 475                 480
189 gtg tta gga gga gtc tac ttc ttt ttc cga ggg att cag tat ttc ctg    2265
190 Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe Leu
191             485                 490                 495
195 cag agg cgg ccg tcg atg aag acc ctg ttt gtg gac agc tac agt gag    2313
196 Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser Glu
197         500                 505                 510
199 atg ctt ttc ttt ctg cag tca ctg ttc atg ctg gcc acc gtg gtg ctg    2361
200 Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val Leu
201     515                 520                 525
203 tac ttc agc cac ctc aag gag tat gtg gct tcc atg gta ttc tcc ctg    2409
204 Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser Leu
205 530                 535                 540                 545
207 gcc ttg ggc tgg acc aac atg ctc tac tac acc cgc ggt ttc cag cag    2457
208 Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln Gln
209                 550                 555                 560
211 atg ggc atc tat gcc gtc atg ata gag aag atg atc ctg aga gac ctg    2505
212 Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp Leu
213             565                 570                 575
215 tgc cgt ttc atg ttt gtc tac atc gtc ttc ttg ttc ggg ttt tcc aca    2553
216 Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser Thr
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221     595                 600                 605
223 gag tcc acg tcg cac agg tgg cgg ggg cct gcc tgc agg ccc ccc gat   2649
224 Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro Asp
225 610                 615                 620                 625
227 agc tcc tac aac agc ctg tac tcc acc tgc ctg gag ctg ttc aag ttc   2697
228 Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
229                 630                 635                 640
233 acc atc ggc atg ggc gac ctg gag ttc act gag aac tat gac ttc aag   2745
234 Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys
235             645                 650                 655
237 gct gtc ttc atc atc ctg ctg ctg gcc tat gta att ctc acc tac atc   2793
238 Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
239         660                 665                 670
241 ctc ctg ctc aac atg ctc atc gcc ctc atg ggt gag act gtc aac aag   2841
242 Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn Lys
243     675                 680                 685
245 atc gca cag gag agc aag aac atc tgg aag ctg cag aga gcc atc acc   2889
246 Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
247 690                 695                 700                 705
249 atc ctg gac acg gag aag agc ttc ctt aag tgc atg agg aag gcc ttc   2937
250 Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala Phe
251                 710                 715                 720
253 cgc tca ggc aag ctg ctg cag gtg ggg tac aca cct gat ggc aag gac   2985
254 Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys Asp
255             725                 730                 735
257 gac tac cgg tgg tgc ttc agg gtg gac gag gtg aac tgg acc acc tgg   3033
258 Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr Trp
259         740                 745                 750
261 aac acc aac gtg ggc atc atc aac gaa gac ccg ggc aac tgt gag ggc   3081
262 Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly
263     755                 760                 765
265 gtc aag cgc acc ctg agc ttc tcc ctg cgg tca agc aga gtt tca ggc   3129
266 Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser Gly
267 770                 775                 780                 785
271 aga cac tgg aag aac ttt gcc ctg gtc ccc ctt tta aga gag gca agt   3177
272 Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala Ser
273                 790                 795                 800
275 gct cga gat agg cag tct gct cag ccc gag gaa gtt tat ctg cga cag   3225
276 Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg Gln
277             805                 810                 815
279 ttt tca ggg tct ctg aag cca gag gac gct gag gtc ttc aag agt cct   3273
280 Phe Ser Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Ser Pro
281         820                 825                 830
283 gcc gct tcc ggg gag aag tga ggacgtcacg cagacagcac tgtcaacact      3324
284 Ala Ala Ser Gly Glu Lys
285     835
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Output Set: **N:\CRF3\10112001\I857123.raw**

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289 cagcctggcc tggtctgtgc ctgcccagca tgttcccaaa tctgtgctgg acaagctgtg 3444
291 ggaagcgttc ttggaagcat ggggagtgat gtacatccaa ccgtcactgt ccccaagtga 3504
293 atctcctaac agactttcag gtttttactc actttactaa acagtttgga tggtcagtct 3564
295 ctactgggac atgttaggcc cttgttttct ttgattttat tcttttctgt gagacagagt 3624
297 tcactcttgt tgcccaggct ggagtgcagt ggtgtgatct tggctcactg caacctctgc 3684
299 tcccgggttc aagcgattct tctgcttcag tctcccaagt agcttggatt acaggtgagc 3744
301 actaccacgc ccggctaatt tttgtatttt taatagagac ggggtttcac catgttggcc 3804
303 aggctggtct cgaactcttg acctcaggtg atctgcccgc cttggcctcc caaagtgctg 3864
305 ggattacagg tgtgagccgc tgcgctcggc cttctttgat tttatattat taggagcaaa 3924
307 agtaaatgaa gcccaggaaa acacctttgg gaacaaactc ttcctttgat ggaaaatgca 3984
311 gaggcccttc ctctctgtgc cgtgcttgct cctcttacct gcccgggtgg tttgggggtg 4044
313 ttggtgtttc ctccctggag aagatggggg aggctgtccc actcccagct ctggcagaat 4104
315 caagctgttg cagcagtgcc ttcttcatcc ttccttacga tcaatcacag tctccagaag 4164
317 atcagctcaa ttgctgtgca ggttaaaact acagaaccac atcccaaagg tacctggtaa 4224
319 gaatgtttga aagatcttcc atttctagga accccagtcc tgcttctccg caatggcaca 4284
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323 aaaaaaaaaa aaaaaaaaaa a                                           4365
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327 <211> LENGTH: 839
328 <212> TYPE: PRT
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331 <400> SEQUENCE: 2
332 Met Lys Lys Trp Ser Ser Thr Asp Leu Gly Ala Ala Ala Asp Pro Leu
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335 Gln Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg
336              20                  25                  30
338 Pro Pro Pro Ala Lys Pro Gln Leu Ser Thr Ala Lys Ser Arg Thr Arg
339          35                  40                  45
341 Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
342      50                  55                  60
344 His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
345  65                  70                  75                  80
347 Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
348                  85                  90                  95
351 Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
352             100                 105                 110
354 Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
355         115                 120                 125
357 Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
358     130                 135                 140
360 Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
361 145                 150                 155                 160
363 Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
364                 165                 170                 175
366 Leu Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn
367             180                 185                 190
369 Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile
370         195                 200                 205
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VERIFICATION SUMMARY
PATENT APPLICATION: US/09/857,123

DATE: 10/11/2001
TIME: 10:54:47

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L:13 M:270 C: Current Application Number differs, Replaced Application Number
L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date

PCT09

RAW SEQUENCE LISTING DATE: 08/30/2001
PATENT APPLICATION: **US/09/857,123** TIME: 07:44:20

Input Set : **A:\ES.txt**
Output Set: **N:\CRF3\08302001\I857123.raw**

Does Not Comply
Corrected Diskette Needed

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      4 <110> APPLICANT: Glaxo Group Ltd
      5       Tate, Simon N
      6       Delany, Natalie S
      7       Sanseau, P
      9 <120> TITLE OF INVENTION: Novel Receptors
     11 <130> FILE REFERENCE: PG3606
C--> 13 <140> CURRENT APPLICATION NUMBER: US/09/857,123
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     16 <150> PRIOR APPLICATION NUMBER: GB 9826359.3
     17 <151> PRIOR FILING DATE: 1998-12-01
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ERRORED SEQUENCES

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VERIFICATION SUMMARY DATE: 08/30/2001
PATENT APPLICATION: **US/09/857,123** TIME: 07:44:21

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L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date
L:1772 M:254 E: No. of Bases conflict, LENGTH:Input:1 Counted:20 SEQ:40

Claims

1. An isolated human vanilloid receptor (hVR) protein or a variant thereof.

2. An isolated human vanilloid receptor (hVR) protein according to claim 1 which is hVR1 or a variant thereof.

3. An isolated human vanilloid receptor (hVR) protein according to claim 1 which is hVR3 or a variant thereof.

4. An isolated human vanilloid receptor (hVR) protein according to claim 2 having an amino acid sequence as shown in Figure 3.

5. An isolated human vanilloid receptor (hVR) protein according to claim 3 having an amino acid sequence as shown in Figure 18.

6. A nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

7. A nucleotide sequence according to claim 6 encoding for an hVR1 protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

8. A nucleotide sequence according to claim 6 encoding for an hVR3 protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

9. A nucleotide sequence according to claim 6 which is a cDNA sequence.

10. A nucleotide sequence according to claim 7 which is a cDNA sequence

11. A nucleotide sequence according to claim 8 which is a cDNA sequence

12. A nucleotide sequence according to claim 7 as shown in Figure 2.

13. A nucleotide sequence according to claim 8 as shown in Figure 17.

14. An expression vector comprising a nucleotide sequence according to any one of claims 6 to 13, which is capable of expressing an hVR protein or a variant thereof.

15. An expression vector according to claim 14 which is capable of expressing an hVR1 protein or a variant thereof.

16. An expression vector according to claim 14 which is capable of expressing an hVR3 protein or a variant thereof.

17. A stable cell line comprising an expression vector according to claim 14.

18. A stable cell line comprising an expression vector according to claim 15.

19. A stable cell line comprising an expression vector according to claim 16.

20. A stable cell line according to claim 17 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

21. A stable cell line according to claim 18 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

22. A stable cell line according to claim 19 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

23. An antibody specific for a human vanilloid receptor (hVR) protein or a variant thereof as claimed in any one of claims 1 to 5.

24. An antibody according to claim 23 which is specific for hVR1 or a variant thereof.

25. An antibody according to claim 23 which is specific for hVR3 or a variant thereof.

26. A method for identification of a compound which exhibits hVR modulating activity comprising contacting a human vanilloid receptor (hVR) protein or a variant thereof according to any one of claims 1 to 5 with a test compound and detecting modulating activity or inactivity.

27. A compound which modulates hVR activity, identifiable by a method according to claim 26.

28. A compound according to claim 27 for use in therapy.

29. The use of a compound according to claim 27 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

30. The use according to claim 28 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

31. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 27.

32. A method according to claim 31 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain,

rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

33. A compound which modulates hVR activity, identifiable by a method according to claim 26, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine.

34. A compound according to claim 33 for use in therapy.

35. The use of a compound according to claim 33 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

36. The use according to claim 35 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

37. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 33.

38. A method according to claim 37 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a

urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

39. A compound identified by the method according to claim 26.

40. A compound according to claim 39 for use in therapy.

41. The use of a compound according to claim 39 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

42. The use according to claim 41 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

43. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 39.

44. A method according to claim 43 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

45. A method of producing an hVR protein or a variant thereof according to any one of claims 1-5 comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or

a variant thereof, under conditions suitable for obtaining expression of the hVR protein or variant thereof.

46. A method of producing an hVR1 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR1 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR1 protein or variant thereof.

47. A method of producing an hVR3 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR3 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR3 protein or variant thereof.

48. A human vanilloid receptor (hVR) protein or a variant thereof for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient

49. A human vanilloid receptor (hVR) protein according to claim 48 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

50. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR1 or a variant thereof.

51. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR3 or a variant thereof.

FIG. 1

ALIGNMENT OF HUMAN VR1 *IN SILICO* DERIVED CLUSTERS WITH RAT VR1

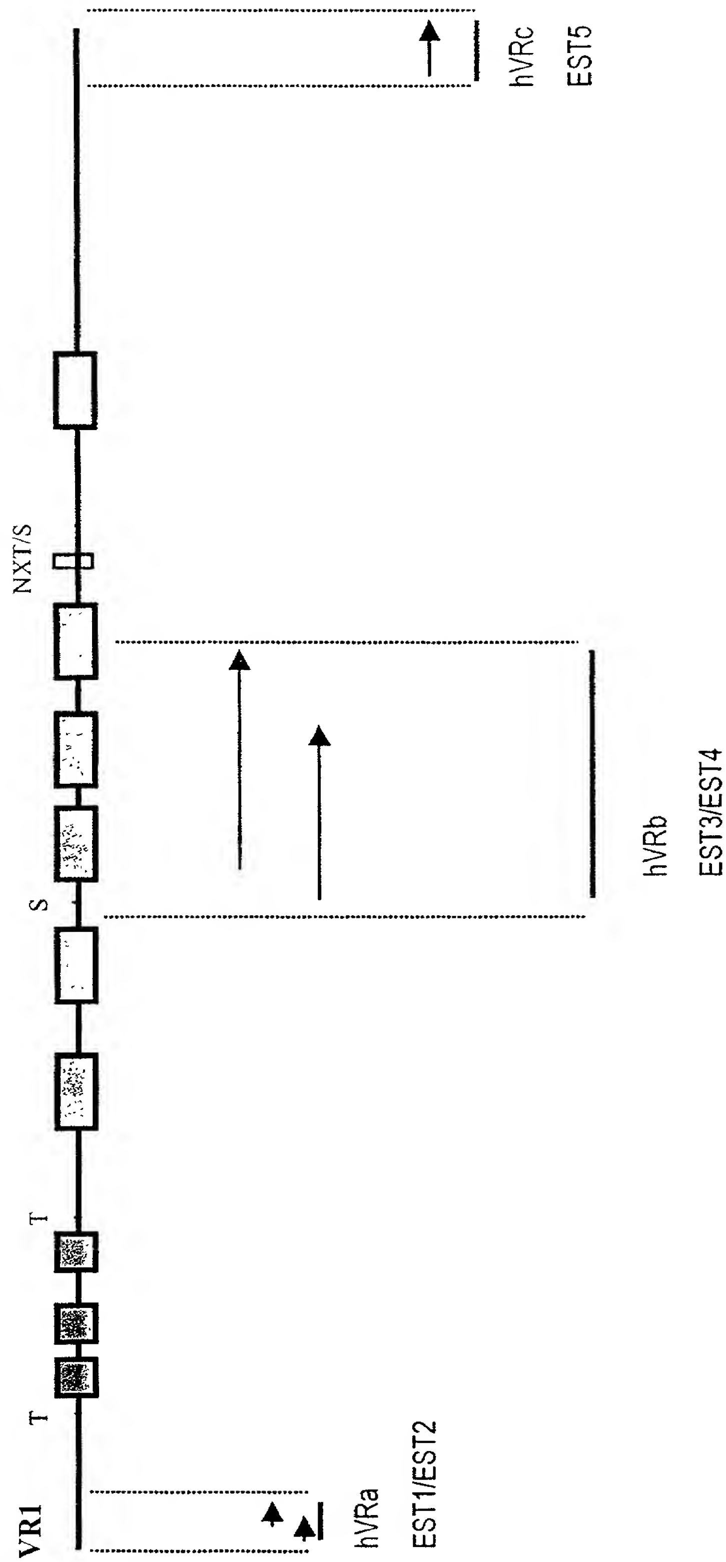

FIG. 2

hVR1 SEQUENCE INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt 1 TO 2517) AND 3'UTR (nt 2518 TO nt 3560)

```
-773  cccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714

-713  aaggccagaagcttgacagatgttgattcataaaaatgcaaaagccaaaatccaaaatct  -654

-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594

-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534

-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474

-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414

-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354

-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294

-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234

-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174

-173  gttctaggggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114

-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54

 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6

   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66

  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126

 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186

 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246

 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
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307 GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT 366

367 GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG 426

427 CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC 486

487 ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG 546

547 CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC 606

607 CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTG 666

667 GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG 726

727 CGGCCTGGATTCTACTTCGGTGAACTGCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTG 786

787 GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC 846

847 TCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC 906

907 ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG 966

967 ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT 1026

1027 GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG 1086

1087 TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG 1146

1147 TACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC 1206

1207 AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC 1266

1267 CTGCAGGACAAGTGGGACAGATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC 1326

1327 TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCT 1386

1387 CCCTTTAAGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG 1446

FIG. 2CONT'D

```
1447 TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTCAGTATTTCCTGCAGAGGCGGCCGTCG 1506
1507 ATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTC 1566
1567 ATGCTGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA 1626
1627 TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG 1686
1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746
1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806
1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGCCTGCCTGCAGG 1866
1867 CCCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926
1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATC 1986
1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046
2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106
2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGC 2166
2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226
2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286
2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346
2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406
2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466
2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526
2527 cacgcagacagcactgtcaacactgggccttaggagaccccgttgccacgggggctgct 2586
```

FIG.2 CONT'D

```
2587  gagggaacaccagtgctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcc  2646
2647  caaatctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgatgtacat  2706
2707  ccaaccgtcactgtccccaagtgaatctcctaacagactttcaggtttttactcacttta  2766
2767  ctaaacagtttggatggtcagtctctactgggacatgttaggcccttgttttctttgatt  2826
2827  ttattcttttctgtgagacagagttcactcttgttgcccaggctggagtgcagtggtgtg  2886
2887  atcttggctcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtctccc  2946
2947  aagtagcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatag  3006
3007  agacggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgc  3066
3067  ccgccttggcctcccaaagtgctgggattacaggtgtgagccgctgcgctcggccttctt  3126
3127  tgattttatattattaggagcaaaagtaaatgaagcccaggaaaacacctttgggaacaa  3186
3187  actcttcctttgatggaaaatgcagaggcccttcctctctgtgccgtgcttgctcctctt  3246
3247  acctgcccgggtggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg  3306
3307  tcccactcccagctctggcagaatcaagctgttgcagcagtgccttcttcatccttcctt  3366
3367  acgatcaatcacagtctccagaagatcagctcaattgctgtgcaggttaaaactacagaa  3426
3427  ccacatcccaaaggtacctggtaagaatgtttgaaagatcttccatttctaggaacccca  3486
3487  gtcctgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa  3546
3547  acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa  3591
```

FIG. 2CONT'D

FIG. 3

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR1 INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt TO 2517) AND 3'UTR (nt 2518 TO nt 3560)

```
-773  cccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714

-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654

-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594

-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534

-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474

-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414

-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354

-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294

-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234

-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174

-173  gttctaggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114

-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54

 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6
   1                                                        M  K    2

   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
   3  K  W  S  S  T  D  L  G  A  A  A  D  P  L  Q  K  D  T  C  P    22

  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
  23  D  P  L  D  G  D  P  N  S  R  P  P  P  A  K  P  Q  L  S  T    42

 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186
  43  A  K  S  R  T  R  L  F  G  K  G  D  S  E  E  A  F  P  V  D    62

 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246
  63  C  P  H  E  E  G  E  L  D  S  C  P  T  I  T  V  S  P  V  I    82

 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
  83  T  I  Q  R  P  G  D  G  P  T  G  A  R  L  L  S  Q  D  S  V    102

 307  GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT  366
 103  A  A  S  T  E  K  T  L  R  L  Y  D  R  R  S  I  F  E  A  V    122

 367  GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG  426
 123  A  Q  N  N  C  Q  D  L  E  S  L  L  L  F  L  Q  K  S  K  K    142

 427  CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC  486
 143  H  L  T  D  N  E  F  K  D  P  E  T  G  K  T  C  L  L  K  A    162

 487  ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG  546
```

```
163   M  L  N  L  H  D  G  Q  N  T  T  I  P  L  L  L  E  I  A  R    182

547   CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC  606
183   Q  T  D  S  L  K  E  L  V  N  A  S  Y  T  D  S  Y  Y  K  G    202

607   CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTG  666
203   Q  T  A  L  H  I  A  I  E  R  R  N  M  A  L  V  T  L  L  V    222

667   GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG  726
223   E  N  G  A  D  V  Q  A  A  A  H  G  D  F  F  K  K  T  K  G    242

727   CGGCCTGGATTCTACTTCGGTGAACTGCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTG  786
243   R  P  G  F  Y  F  G  E  L  P  L  S  L  A  A  C  T  N  Q  L    262

787   GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC  846
263   G  I  V  K  F  L  L  Q  N  S  W  Q  T  A  D  I  S  A  R  D    282

847   TCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC  906
283   S  V  G  N  T  V  L  H  A  L  V  E  V  A  D  N  T  A  D  N    302

907   ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG  966
303   T  K  F  V  T  S  M  Y  N  E  I  L  I  L  G  A  K  L  H  P    322

967   ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT  1026
323   T  L  K  L  E  E  L  T  N  K  K  G  M  T  P  L  A  L  A  A    342

1027  GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG  1086
343   G  T  G  K  I  G  V  L  A  Y  I  L  Q  R  E  I  Q  E  P  E    362

1087  TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG  1146
363   C  R  H  L  S  R  K  F  T  E  W  A  Y  G  P  V  H  S  S  L    382

1147  TACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC  1206
383   Y  D  L  S  C  I  D  T  C  E  K  N  S  V  L  E  V  I  A  Y    402

1207  AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC  1266
403   S  S  S  E  T  P  N  R  H  D  M  L  L  V  E  P  L  N  R  L    422

1267  CTGCAGGACAAGTGGGACAGATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC  1326
423   L  Q  D  K  W  D  R  F  V  K  R  I  F  Y  F  N  F  L  V  Y    442

1327  TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCT  1386
443   C  L  Y  M  I  I  F  T  M  A  A  Y  Y  R  P  V  D  G  L  P    462

1387  CCCTTTAAGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG  1446
463   P  F  K  M  E  K  I  G  D  Y  F  R  V  T  G  E  I  L  S  V    482

1447  TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTCAGTATTTCCTGCAGAGGCGGCCGTCG  1506
483   L  G  G  V  Y  F  F  F  R  G  I  Q  Y  F  L  Q  R  R  P  S    502

1507  ATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTC  1566
503   M  K  T  L  F  V  D  S  Y  S  E  M  L  F  F  L  Q  S  L  F    522

1567  ATGCTGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA  1626
523   M  L  A  T  V  V  L  Y  F  S  H  L  K  E  Y  V  A  S  M  V    542

1627  TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG  1686
```

FIG. 3CONT'D

543 F S L A L G W T N M L Y Y T R G F Q Q M 562

1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746
563 G I Y A V M I E K M I L R D L C R F M F 582

1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806
583 V Y I V F L F G F S T A V V T L I E D G 602

1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGCCTGCCTGCAGG 1866
603 K N D S L P S E S T S H R W R G P A C R 622

1867 CCCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926
623 P P D S S Y N S L Y S T C L E L F K F T 642

1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATC 1986
643 I G M G D L E F T E N Y D F K A V F I I 662

1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046
663 L L L A Y V I L T Y I L L L N M L I A L 682

2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106
683 M G E T V N K I A Q E S K N I W K L Q R 702

2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGC 2166
703 A I T I L D T E K S F L K C M R K A F R 722

2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226
723 S G K L L Q V G Y T P D G K D D Y R W C 742

2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286
743 F R V D E V N W T T W N T N V G I I N E 762

2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346
763 D P G N C E G V K R T L S F S L R S S R 782

2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406
783 V S G R H W K N F A L V P L L R E A S A 802

2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466
803 R D R Q S A Q P E E V Y L R Q F S G S L 822

2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526
823 K P E D A E V F K S P A A S G E K 839

2527 cacgcagacagcactgtcaacactgggccttaggagaccccgttgccacgggggctgct 2586

2587 gagggaacaccagtgctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcc 2646

2647 caaatctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgatgtacat 2706

2707 ccaaccgtcactgtccccaagtgaatctcctaacagactttcaggtttttactcacttta 2766

2767 ctaaacagtttggatggtcagtctctactgggacatgttaggcccttgttttctttgatt 2826

2827 ttattcttttctgtgagacagagttcactcttgttgcccaggctggagtgcagtggtgtg 2886

2887 atcttggctcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtctccc 2946

FIG. 3CONT'D

2947 aagtagcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatag 3006
3007 agacggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgc 3066
3067 ccgccttggcctcccaaagtgctgggattacaggtgtgagccgctgcgctcggccttctt 3126
3127 tgattttatattattaggagcaaaagtaaatgaagcccaggaaaacacctttgggaacaa 3186
3187 actcttcctttgatggaaaatgcagaggcccttcctctctgtgccgtgcttgctcctctt 3246
3247 acctgcccgggtggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg 3306
3307 tcccactcccagctctggcagaatcaagctgttgcagcagtgccttcttcatccttcctt 3366
3367 acgatcaatcacagtctccagaagatcagctcaattgctgtgcaggttaaaactacagaa 3426
3427 ccacatcccaaaggtacctggtaagaatgtttgaaagatcttccatttctaggaacccca 3486
3487 gtcctgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa 3546
3547 acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 3591

FIG. 3CONT'D

FIG. 4

AMINO ACID SEQUENCE OF hVR1

1 MKKWSSTDLG AAADPLQKDT CPDPLDGDPN SRPPPAKPQL STAKSRTRLF

51 GKGDSEEAFP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD

101 SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK

151 DPETGKTCLL KAMLNLHDGQ NTTIPLLLEI ARQTDSLKEL VNASYTDSYY

201 KGQTALHIAI ERRNMALVTL LVENGADVQA AAHGDFFKKT KGRPGFYFGE

251 LPLSLAACTN QLGIVKFLLQ NSWQTADISA RDSVGNTVLH ALVEVADNTA

301 DNTKFVTSMY NEILILGAKL HPTLKLEELT NKKGMTPLAL AAGTGKIGVL

351 AYILQREIQE PECRHLSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI

401 AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKRIFYFNFL VYCLYMIIFT

451 MAAYYRPVDG LPPFKMEKIG DYFRVTGEIL SVLGGVYFFF RGIQYFLQRR

501 PSMKTLFVDS YSEMLFFLQS LFMLATVVLY FSHLKEYVAS MVFSLALGWT

551 NMLYYTRGFQ QMGIYAVMIE KMILRDLCRF MFVYIVFLFG FSTAVVTLIE

601 DGKNDSLPSE STSHRWRGPA CRPPDSSYNS LYSTCLELFK FTIGMGDLEF

651 TENYDFKAVF IILLLAYVIL TYILLLNMLI ALMGETVNKI AQESKNIWKL

701 QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW

751 TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA

801 SARDRQSAQP EEVYLRQFSG SLKPEDAEVF KSPAASGEK*

Key

T/S predicted phosphorylation sites

Transmembrane domains

Ankyrin binding domains

FIG. 5

COMPARISON OF THE AMINO ACID SEQUENCE OF THE RAT (VR1) AND HUMAN (hVR1) VANILLOID PROTEINS.

10 20 30 40 50
VR1 MEQRASLDSEESESPPQENSCLDPPDRDPNCKPPPVKPHIFTTRSRTRLF
hVR1 MKKWSSTDLGAAADPLQKDTCPDPLDGDPNSRPPPAKPQLSTAKSRTRLF

60 70 80 90 100
VR1 GKGDSEEASPLDCPYEEGGLASCPIITVSSVLTIQRPGDGPASVRPSSQD
hVR1 GKGDSEEAFPVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD

110 120 130 140 150
VR1 SVSAG.EKPPRLYDRRSIFDAVAQSNCQELESLLPFLQRSKKRLTDSEFK
hVR1 SVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFK

160 170 180 190 200
VR1 DPETGKTCLLKAMLNLHNGQNDTIALLLDVARKTDSLKQFVNASYTDSYY
hVR1 DPETGKTCLLKAMLNLHDGQNTTIPLLLEIARQTDSLKELVNASYTDSYY

210 220 230 240 250
VR1 KGQTALHIAIERRNMTLVTLLVENGADVQAAANGDFFKKTKGRPGFYFGE
hVR1 KGQTALHIAIERRNMALVTLLVENGADVQAAAHGDFFKKTKGRPGFYFGE

260 270 280 290 300
VR1 LPLSLAACTNQLAIVKFLLQNSWQPADISARDSVGNTVLHALVEVADNTV
hVR1 LPLSLAACTNQLGIVKFLLQNSWQTADISARDSVGNTVLHALVEVADNTV

310 320 330 340 350
VR1 DNTKFVTSMYNEILILGAKLHPTLKLEEITNRKGLTPLALAASSGKIGVL
hVR1 DNTKFVTSMYNEILILGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGVL

360 370 380 390 400
VR1 AYILQREIHEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI
hVR1 AYILQREIQEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI

410 420 430 440 450
VR1 AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFFVYCLYMIIFT
hVR1 AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIFT

460 470 480 490 500
VR1 AAAYYRPVEGLPPYKLKWTVGDYFRVTGEILSVSGGVYFFFRGIQYFLQR
hVR1 MAAYYRPVDGLPPFKMEK.IGDYFRVTGEILSVLGGVYFFFRGIQYFLQR

510 520 530 540 550
VR1 RPSLKSLFVDSYSEILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGW
hVR1 RPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGW

560 570 580 590 600
VR1 TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLI
hVR1 TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLI

610 620 630 640 650
VR1 EDGKNNSLPMESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLE
hVR1 EDGKNDSLPSESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLE

660 670 680 690 700
VR1 FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK
hVR1 FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK

710 720 730 740 750
VR1 LQRAITILDTEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVN
hVR1 LQRAITILDTEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVN

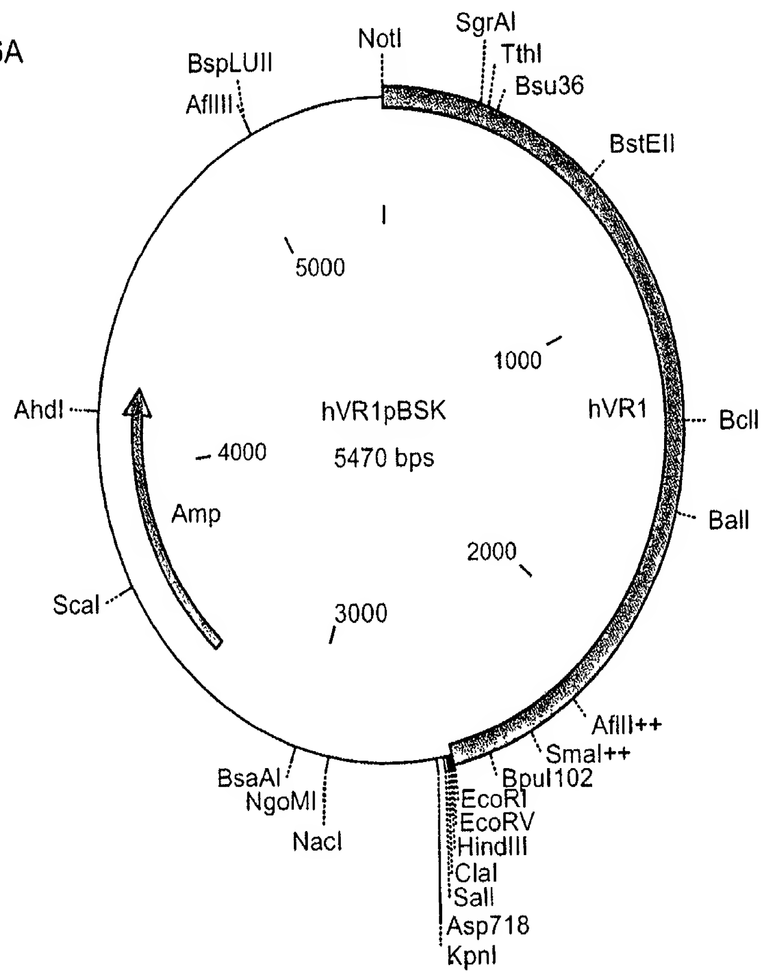
760 770 780 790 800
VR1 WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSGRVSGRNWKNFALVPLLRD
hVR1 WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSSRVSGRHWKNFALVPLLRE

810 820 830
VR1 ASTRDRHATQQEEVQLKHYTGSLKPEDAEVFKDSMVPGEK
hVR1 ASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEK

FIG. 6

FULL-LENGTH hRV1 CLONED INTO (A) pBLUESCRIPT SK(+) (hVR1pBSK) AND (B) pCIN5-NEW (hVR1pCIN5) VIA NotI/EcoRI RESTRICTION SITES.

6A



6B

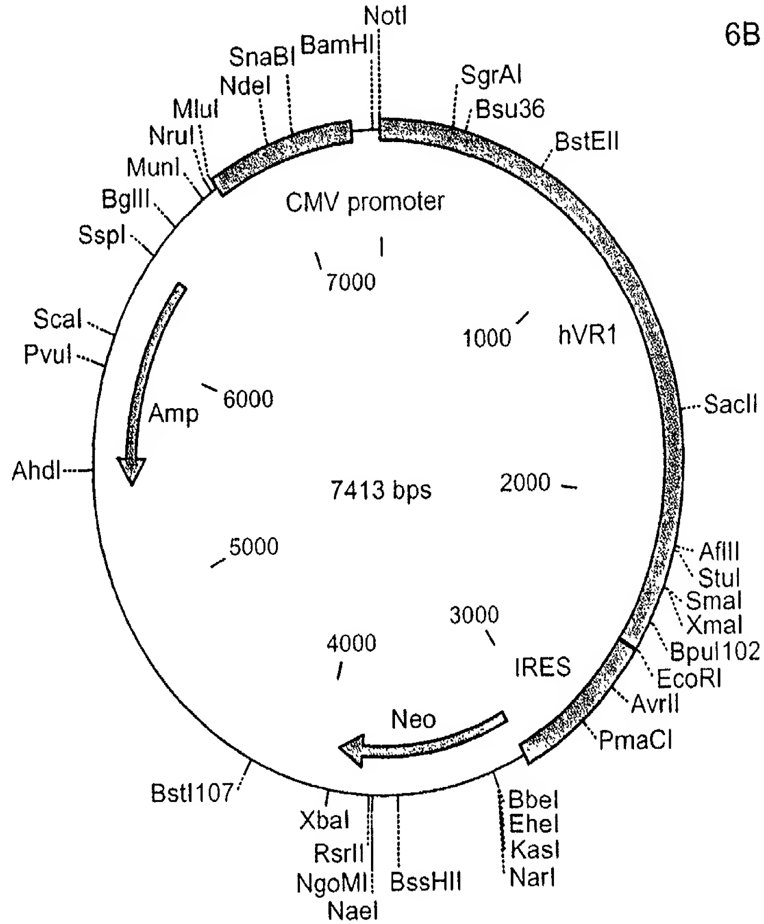
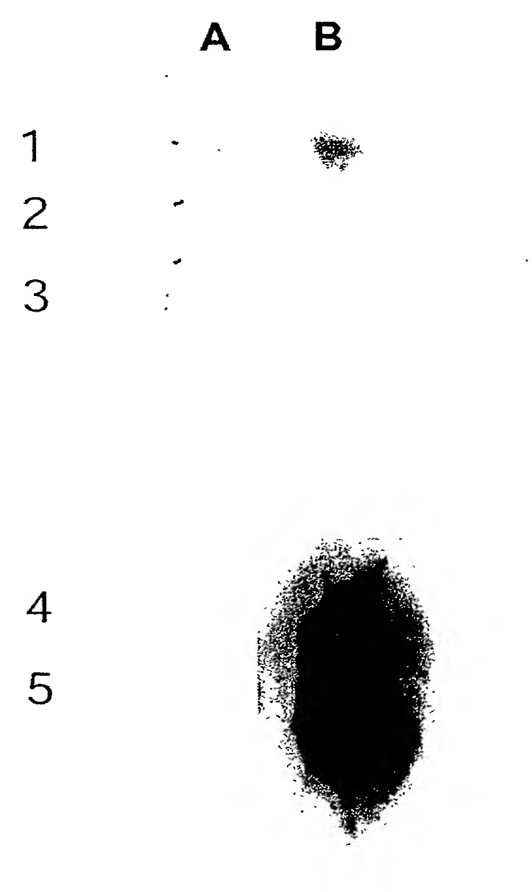


FIG. 7

SLOT HYBRIDISATION WITH hVR1 PROBE



Well
1A hDRG 1B hDRG
2A rDRG

3A Water

4B EST3 clone
5B 260bp Amplicon from Brain cDNA

FIG. 8

WESTERN BLOT PROBED WITH ANTI-hVR1 ANTIBODIES.
ARROW POINTS TO hVR1 SPECIFIC BAND

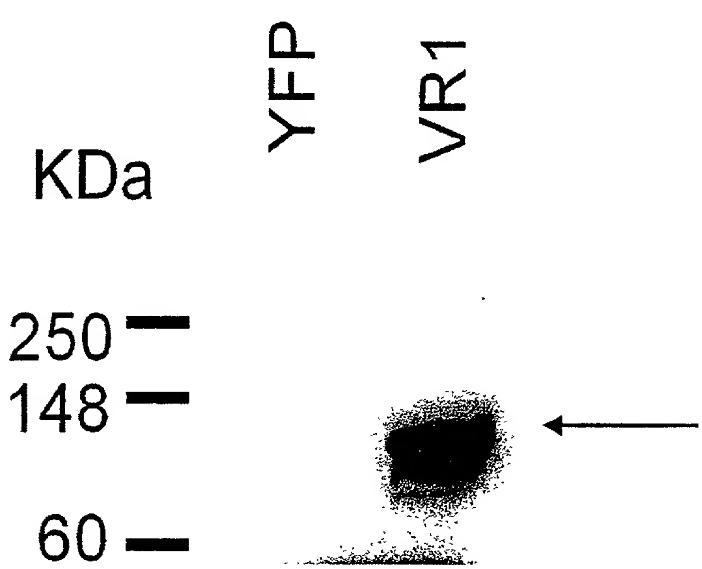


FIG. 9

IN SITU LOCALISATION OF VR1 IN RAT DRG TISSUE SECTIONS. ARROW POINTS TO A VR1 EXPRESSING SMALL DIAMETER (<25µn) NEURONE CELL BODY, MAGNIFICATION USED 147x10.

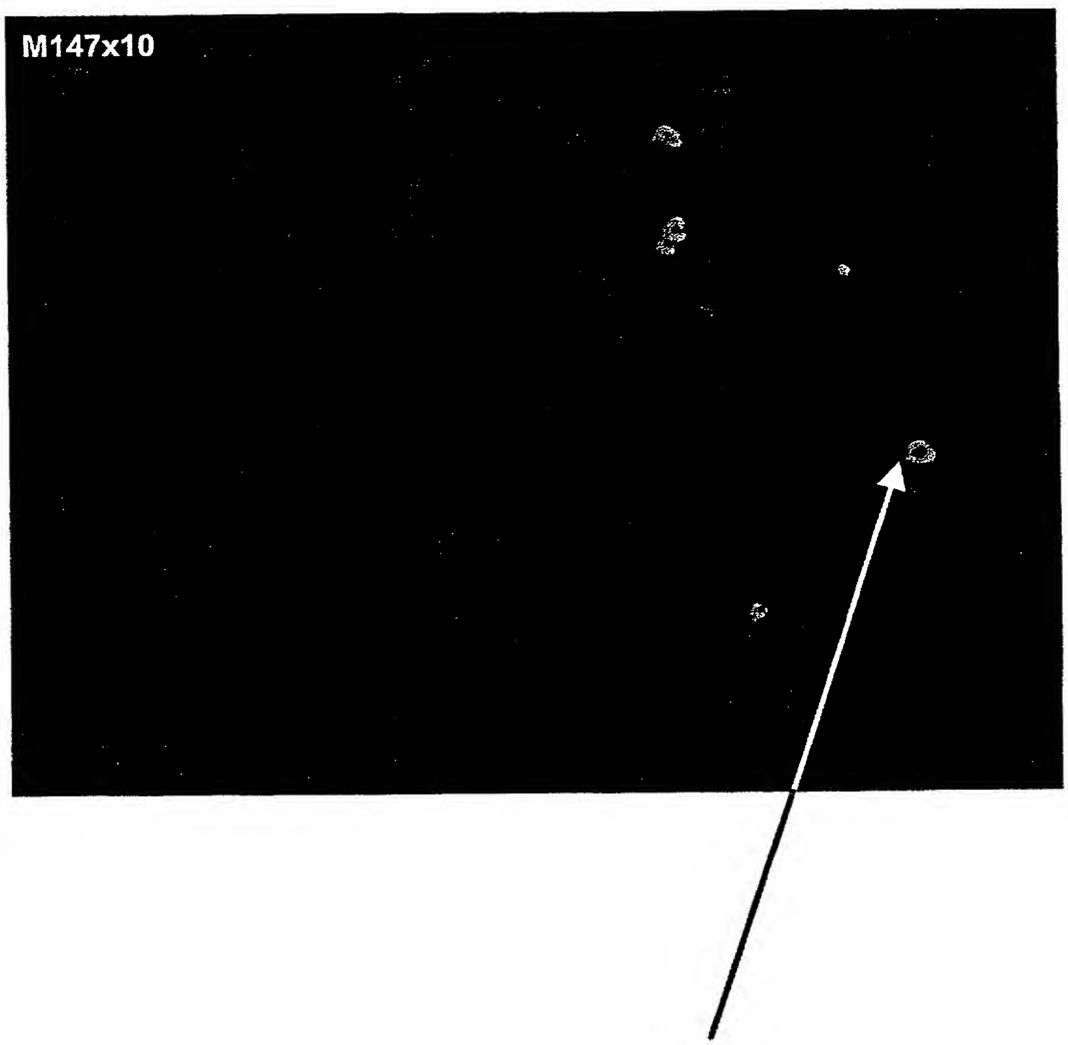
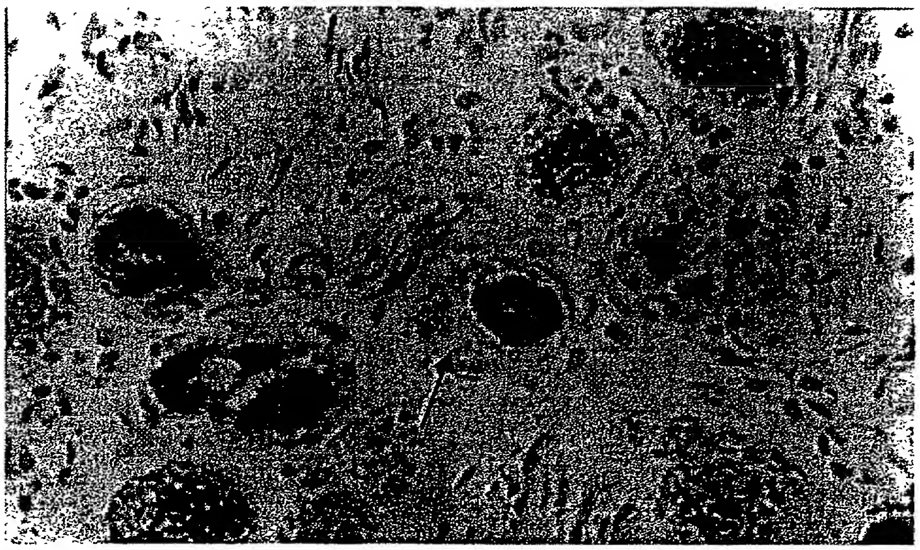


FIG. 10A



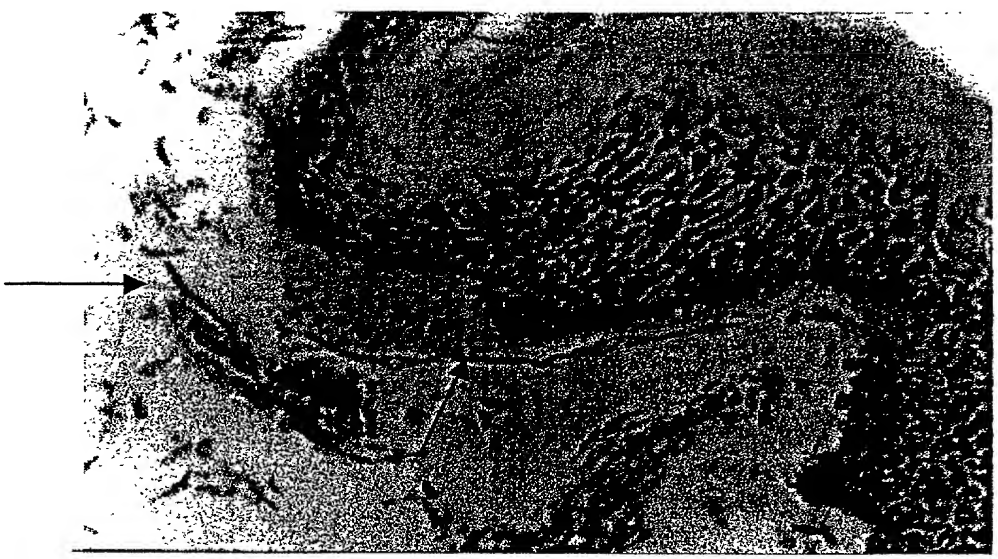


FIG. 10B

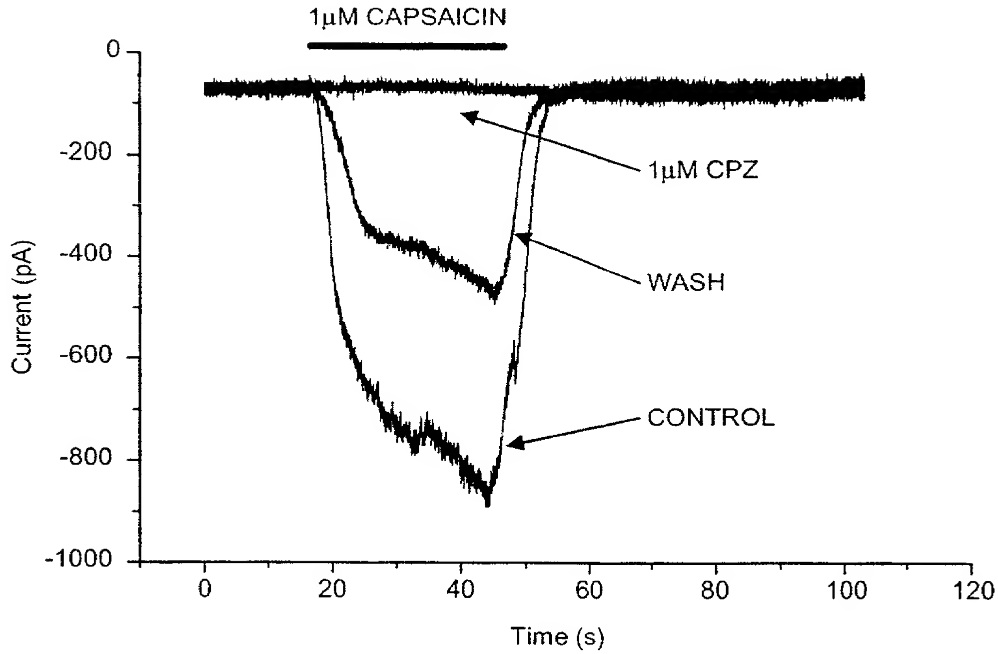
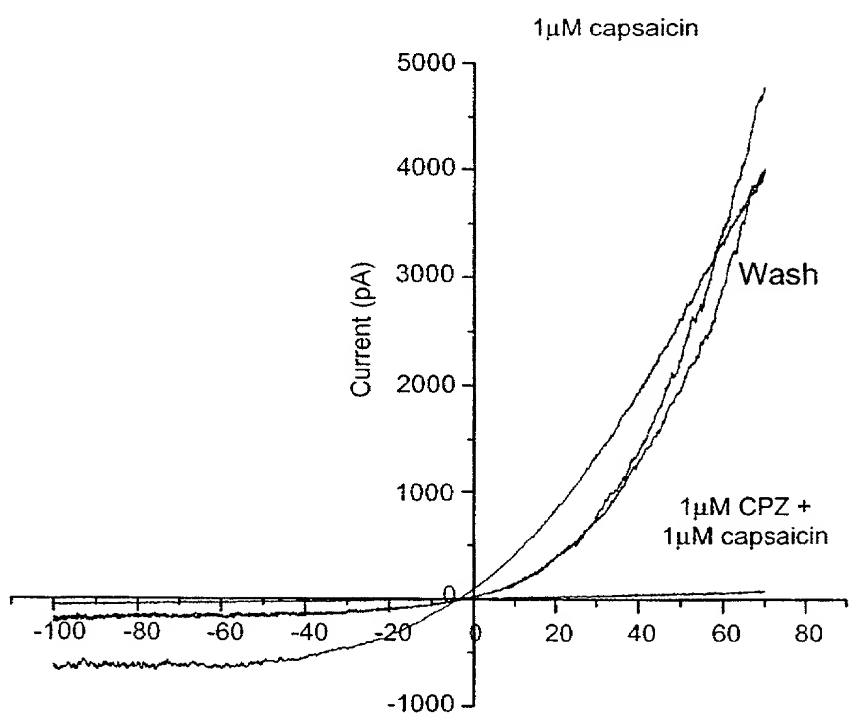


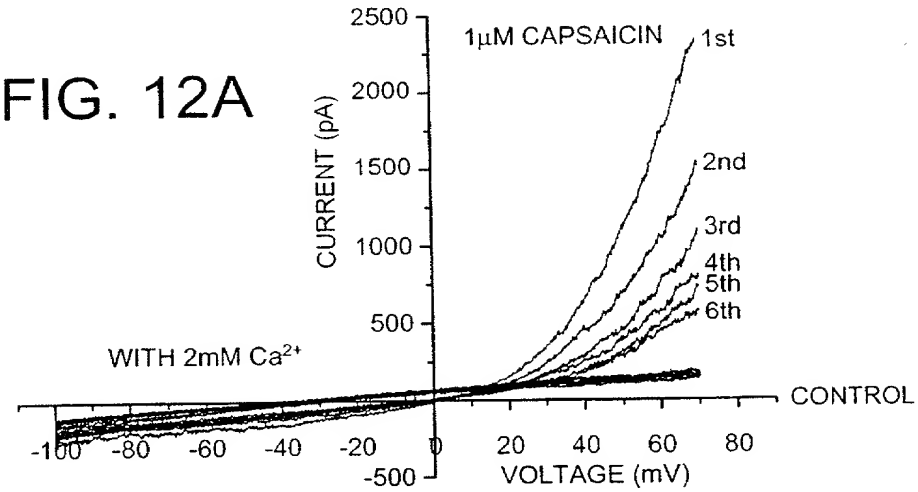
FIG. 11A



SOLUTIONS
OUTSIDE 140mM Na^+ 2mM Ca^{2+}
INSIDE 140mM Cs^+

FIG. 11B

FIG. 12A



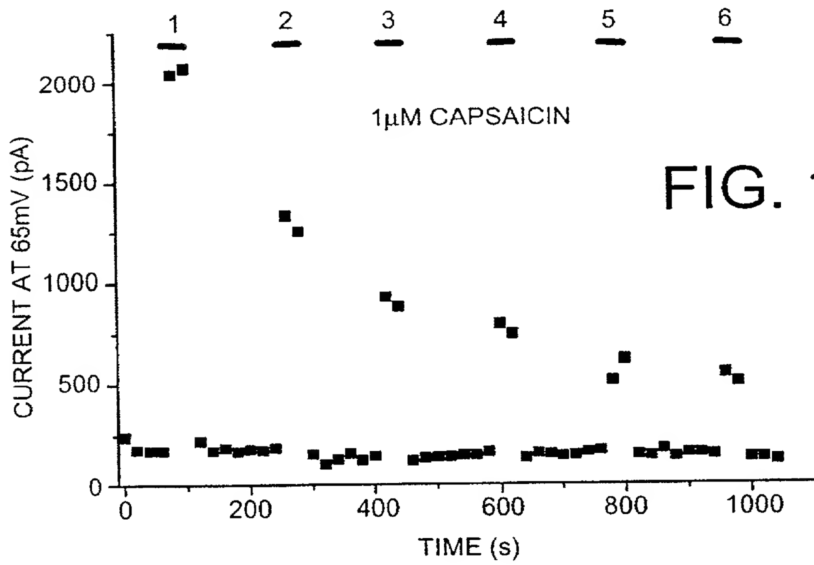


FIG. 12B

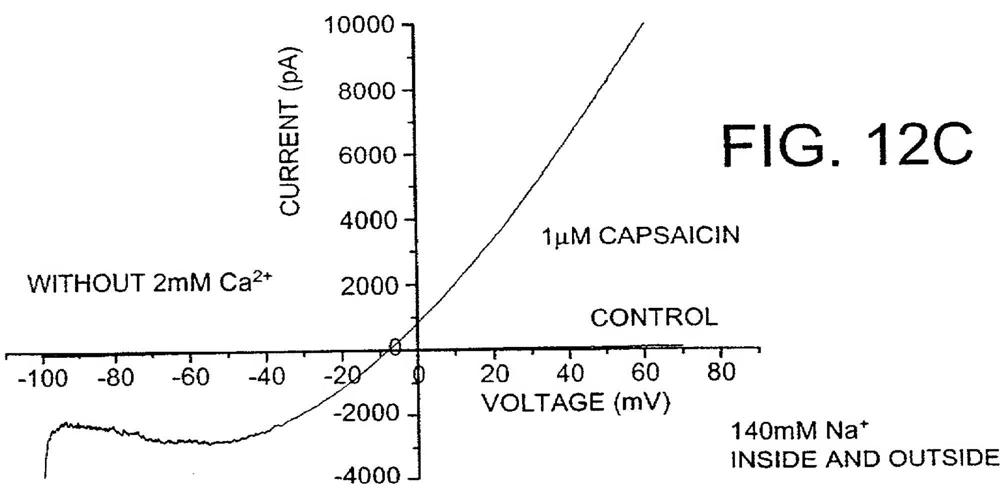
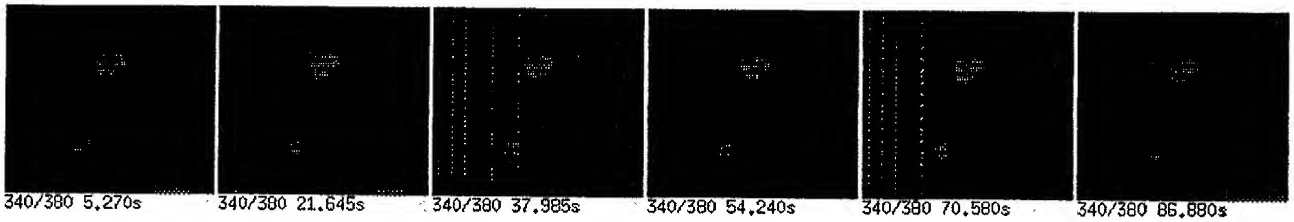


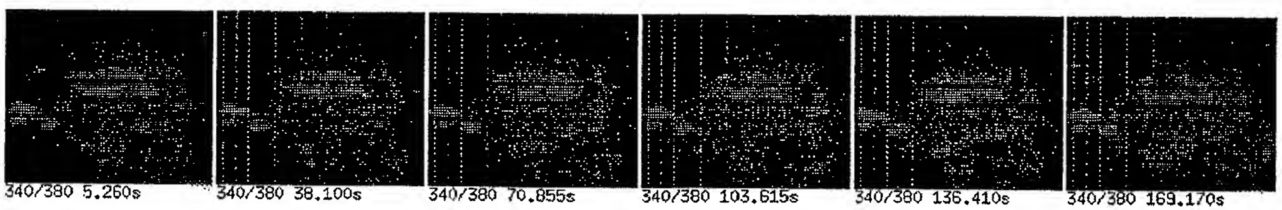
FIG. 12C

13A pCIN5-new in HEK293T, 24hr transient expression, stimulated with 3μM capsaicin at time point 52 secs of time course



13B hVR1pCIN5 in HEK293T, 24hr expression, stimulated with 1μM capsaicin at time point 52 seconds

13C hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 uM capsazepine, stimulated with 1 μM capsaicin at time point 52 seconds of time course



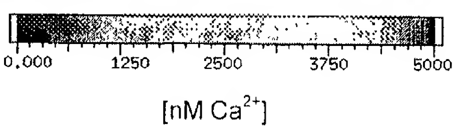
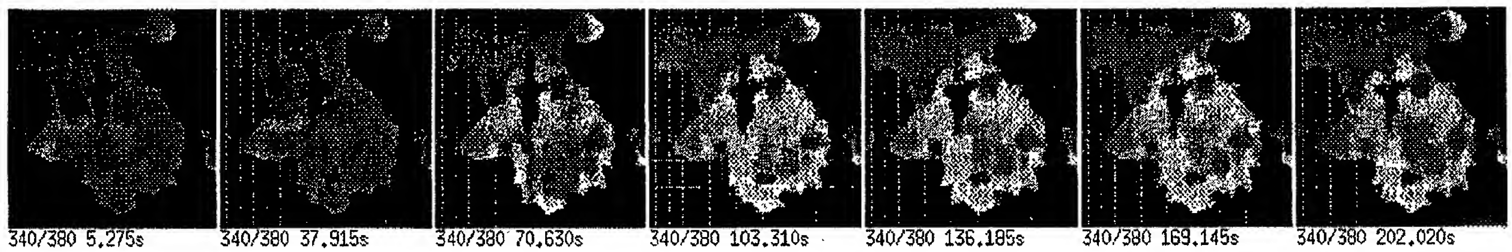


FIG. 13

13D hVR1pCIN5 in HEK293T, 24hr transient expression, stimulated with 10uM anandamide at time point 52 seconds



13E hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation in 10uM capsazepine, stimulated with 10uM anandamide at time point 52 sec

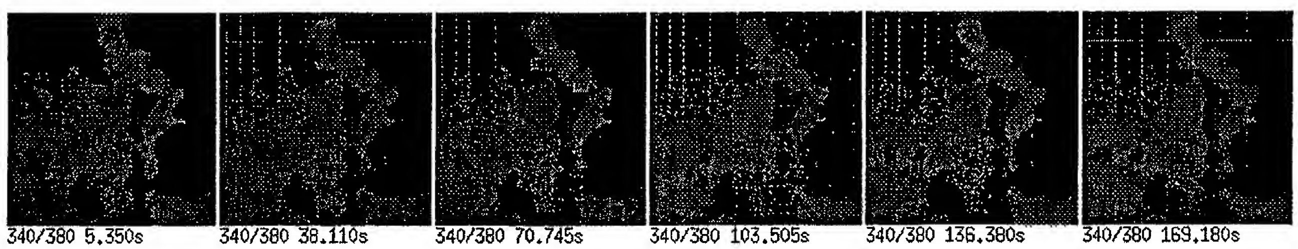


FIG. 13CONT'D

13F hVR1pCIN5 in HEK293T cells, 24hr transient expression, stimulated with 1uM Resiniferatoxin at time point 52 seconds

13G hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 uM capsazepine, stimulated with 1 uM Resiniferatoxin at time point 52 seconds

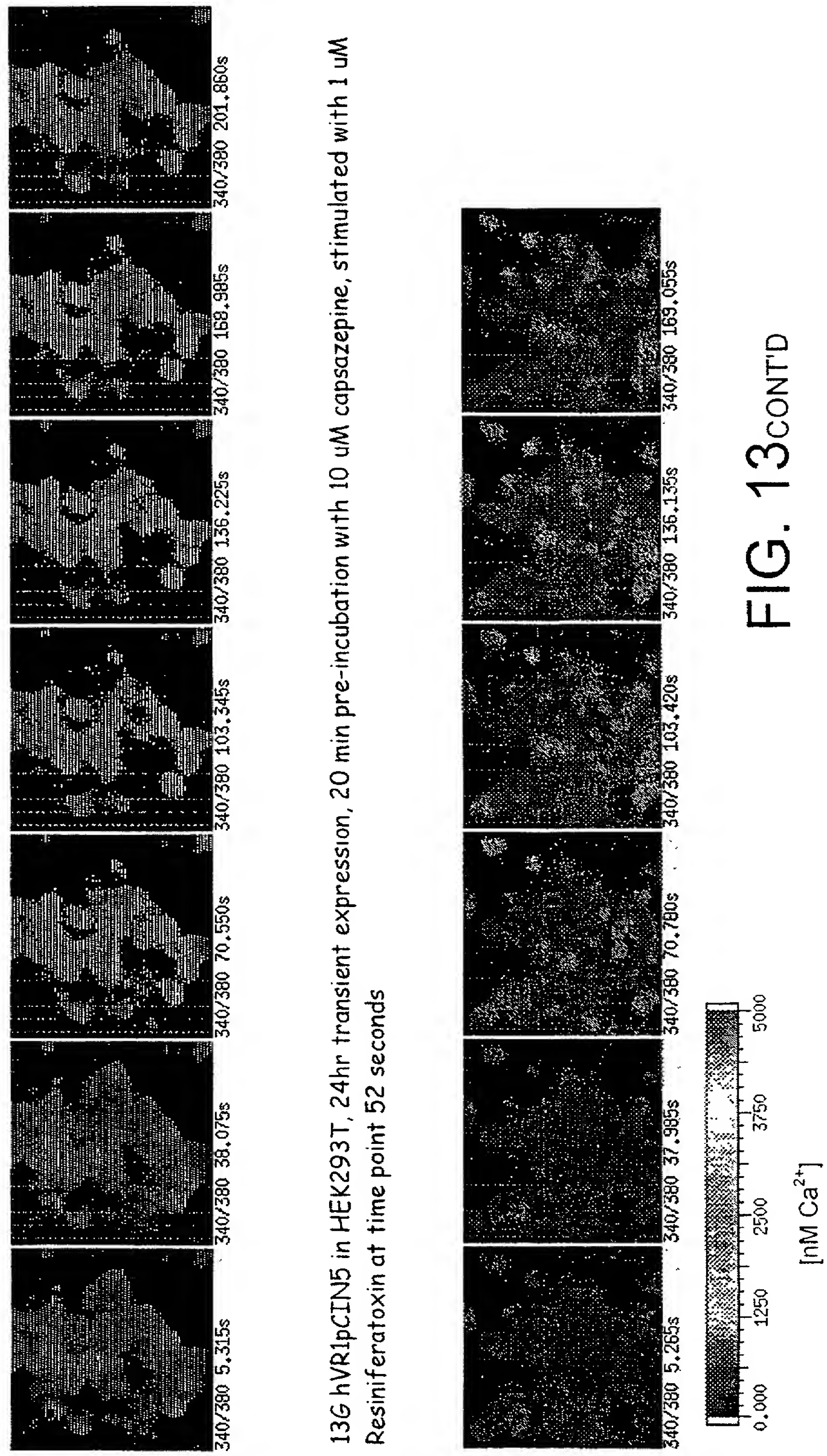
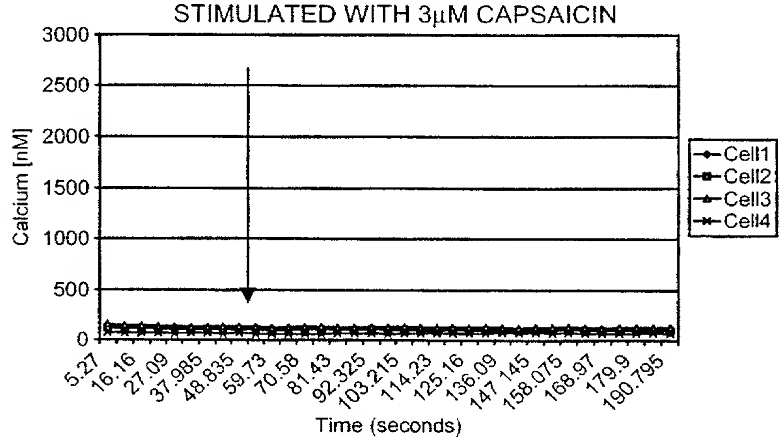


FIG. 13CONT'D

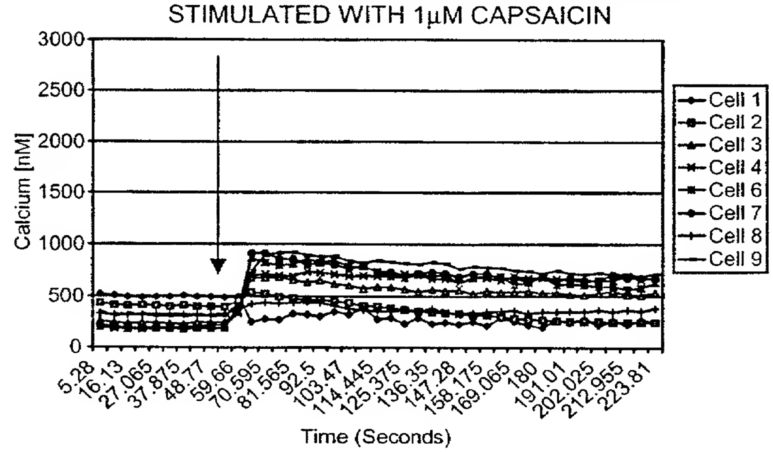
FIG. 14

EXPOSURE OF TRANSFECTED CELLS TO AGONISTS
(ADDITION INDICATED BY ARROW).

14A: pCIN5-NEW IN HEK293T, 24hr TRANSIENT EXPRESSION,
STIMULATED WITH 3μM CAPSAICIN



14B: hVR1pCIN5 IN HEK293T, 24hr EXPRESSION,
STIMULATED WITH 1μM CAPSAICIN



14C: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT
EXPRESSION, 20 MIN PRE-INCUBATION WITH 10μM
CAPSAZEPINE, STIMULATION WITH 1μM CAPSIACIN

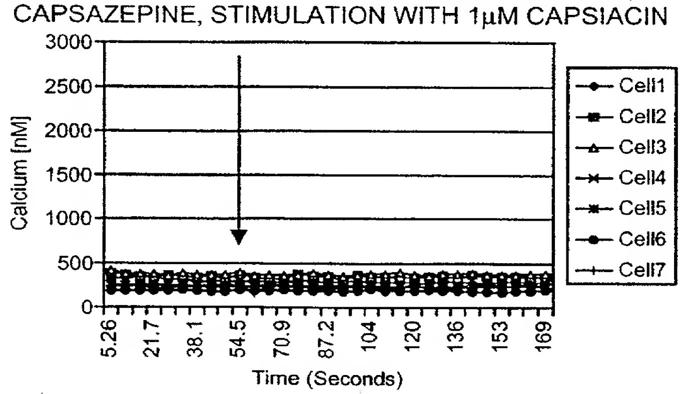
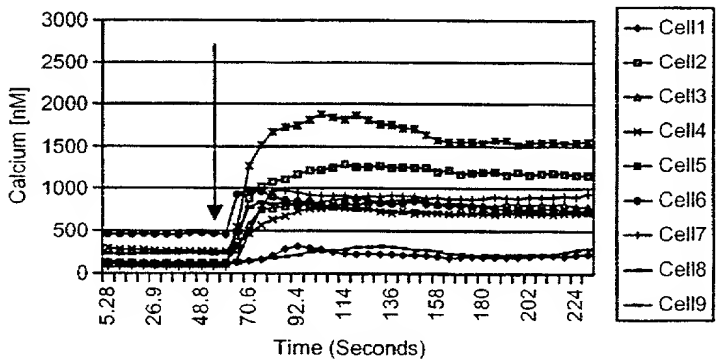


FIG. 14CONT'D

14D: hVR1pCIN5 IN HEK293T, 24hR TRANSIENT EXPRESSION, STIMULATION WITH 10µM ANANDAMIDE



14E: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT EXPRESSION, 20 MIN PRE-INCUBATION IN 10µM CAPAZEPINE, STIMULATED WITH 10µM ANANDAMIDE

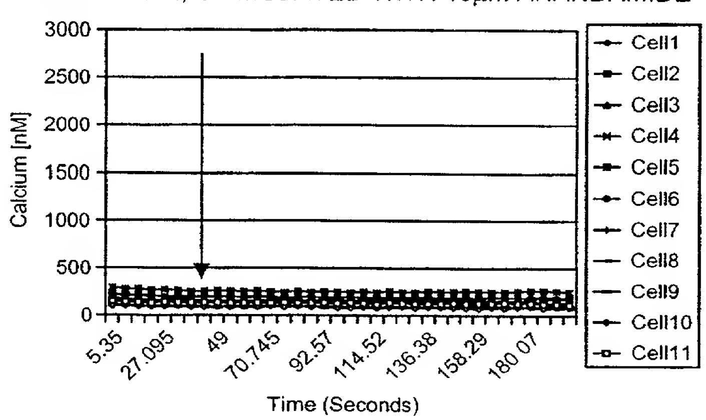
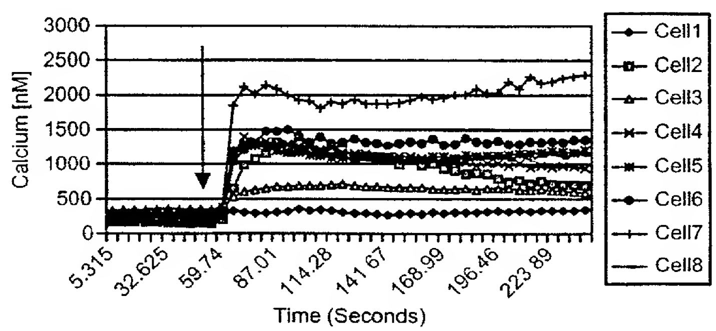
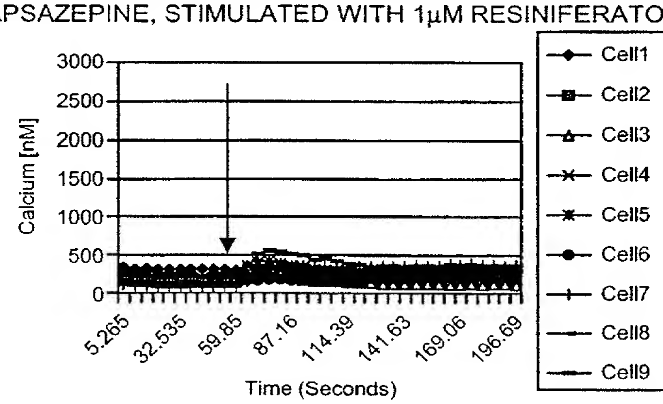


FIG. 14CONT'D

14F: hVR1pCIN5 IN HEK293T CELLS, 24hr TRANSIENT EXPRESSION, STIMULATED WITH 1μM RESINIFERATOXIN



14G: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT EXPRESSION, 20 MIN PRE-INCUBATION WITH 10μM CAPSAZEPINE, STIMULATED WITH 1μM RESINIFERATOXIN



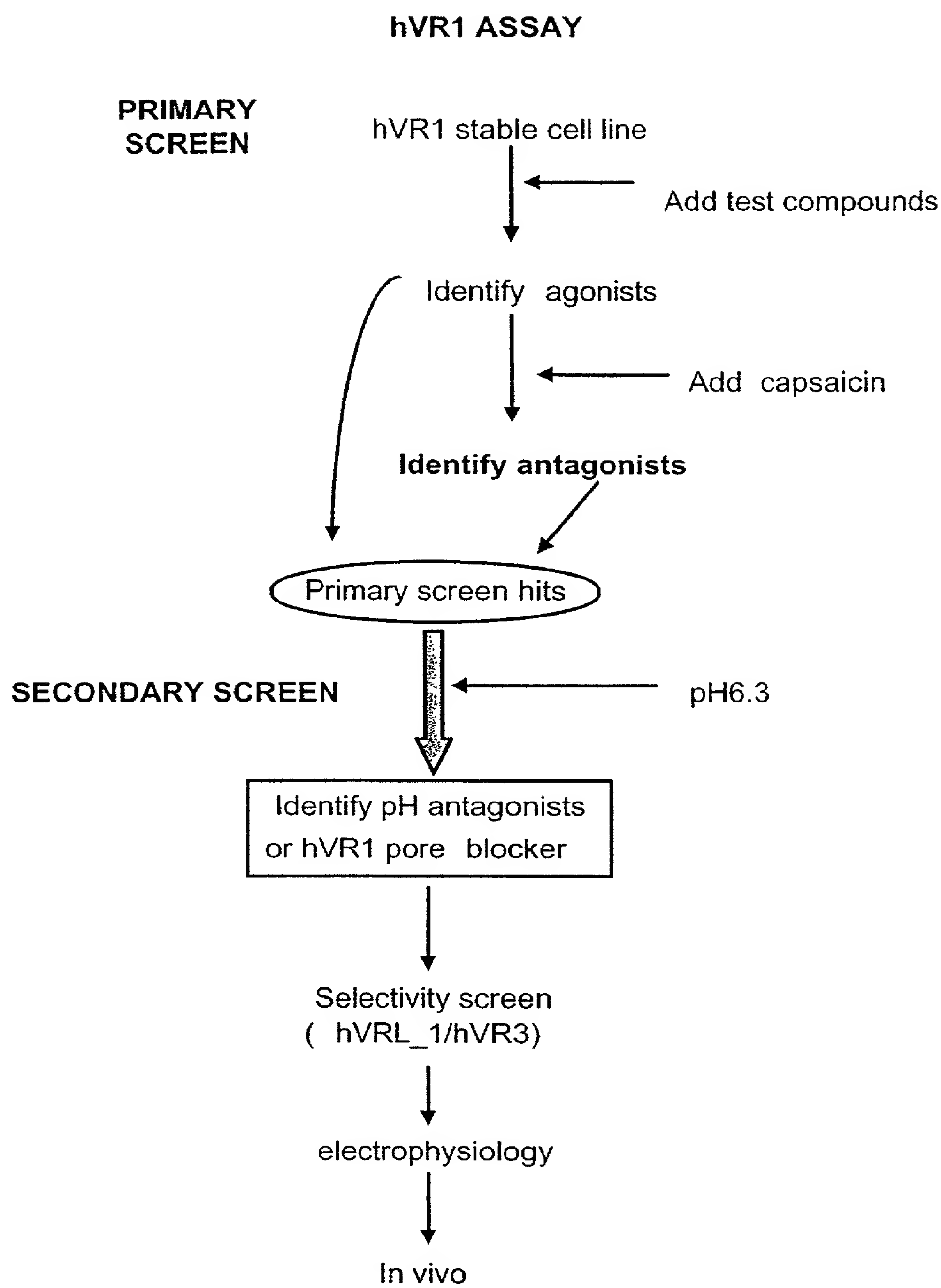

hVR1 ASSAY
PRIMARY SCREEN
hVR1 stable cell line
Add test compounds
Identify agonists
Add capsaicin
Identify antagonists
Primary screen hits
SECONDARY SCREEN
pH6.3
Identify pH antagonists or hVR1 pore blocker
Selectivity screen (hVRL_1/hVR3)
electrophysiology
In vivo

FIG. 15

FIG. 16

ALIGNMENT OF THE HUMAN VR3 *IN SILICO* CLUSTERS WITH RAT VR1

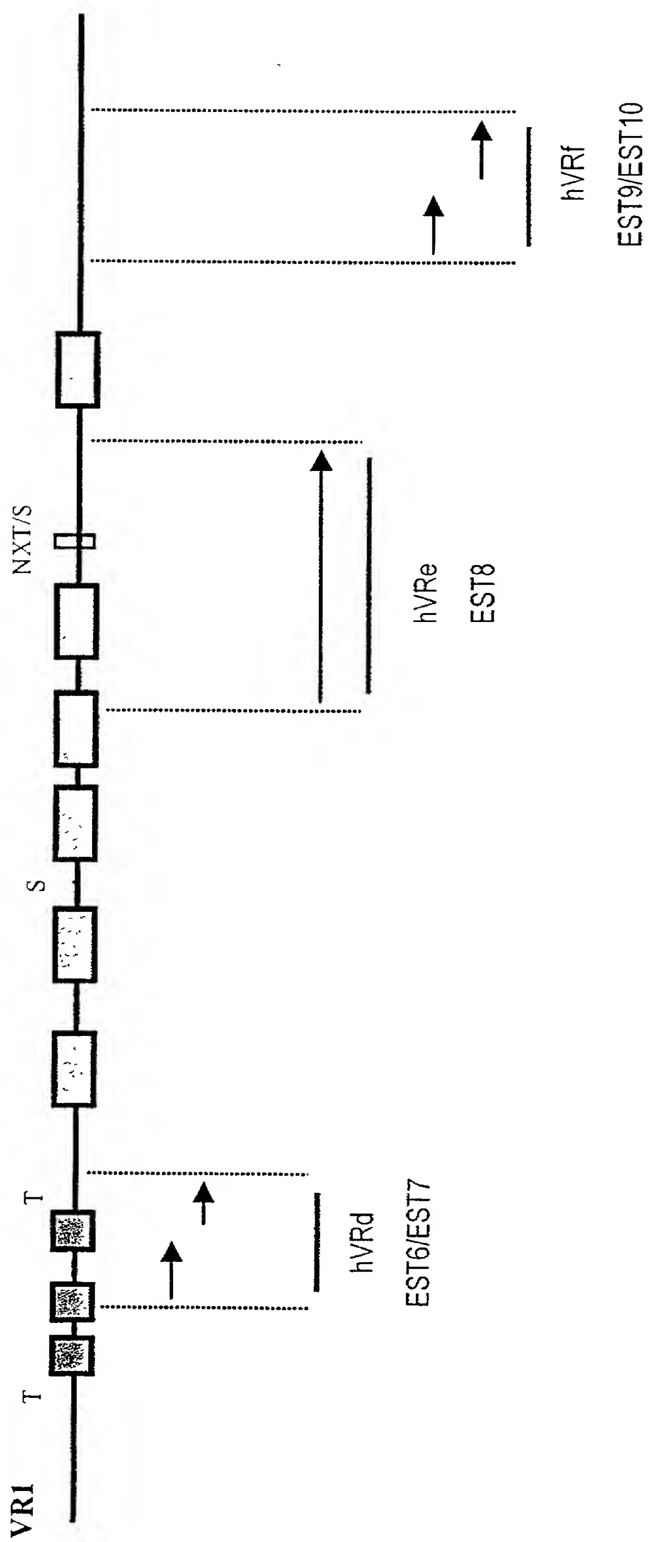


FIG. 17

hVR3 SEQUENCE INCLUDING 5' UTR (nt -686 TO nt 0) CODING REGION (nt1 TO nt 2889), 3'UTR (nt 2890 TO nt 3418)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625
-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565
-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505
-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445
-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385
-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca  -325
-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265
-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205
-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145
-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85
 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25
 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35
  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95
  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155
 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT  215
 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275
 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGGAGGTGGCTGAGCTCCCCGG  335
 336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
```

```
396   TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC   455
456   AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC   515
516   CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA   575
576   GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA   635
636   CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCAAAGCCCCTGCCCC   695
696   TCAGCCGCCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG   755
756   GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCCACAAGAAACGCCT   815
816   AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT   875
876   GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC   935
936   CGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC   995
996   AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAACTTCTCGTGGCCCA   1055
1056  GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGGG   1115
1116  CTACTTCTACTTTGGGGAGCTGCCCCTGTCGCTGGCTGCCTGCACCAACCAGCCCCACAT   1175
1176  TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG   1235
1236  AGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA   1295
1296  GTTTGTTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCCTCTTCCCCGACAG   1355
1356  CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGATGGCTGCCAAGAC   1415
1416  GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG   1475
1476  GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGTATTCCTCGCTTTATGA   1535
```

FIG. 17 CONT'D

1536 CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGATCCTGGTGTACAA 1595

1596 CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG 1655

1656 GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG 1715

1716 TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA 1775

1776 CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC 1835

1836 TGGGGTCCTGTTCTTCTTCACCAACATCAAAGACTTGTTCATGAAGAAATGCCCTGGAGT 1895

1896 GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCCTGGT 1955

1956 GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT 2015

2016 TGCCCTGGTCCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG 2075

2076 GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCCTGCTCGT 2135

2136 CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC 2195

2196 CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG 2255

2256 CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT 2315

2316 GGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGGTCTTCATCATCCTGCTGGT 2375

2376 GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA 2435

2436 GACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGAAGCTGCAGTGGGCCACCAC 2495

2496 CATCCTGGACATTGAGCGCTCCTTCCCCGTATTCCTGAGGAAGGCCTTCCGCTCTGGGGA 2555

2556 GATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACCGCAGGTGGTGCTTCAGGGT 2615

2616 GGATGAGGTGAACTGGTCTCACTGGAACCAGAACTTGGGCATCATCAACGAGGACCCGGG 2675

FIG. 17 CONT'D

```
2676  CAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCGTGGGCCGCCTCCGCAGGGA  2735
2736  TCGCTGGTCCTCGGTGGTACCCCGCGTGGTGGAACTGAACAAGAACTCGAACCCGGACGA  2795
2796  GGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCCGCTGCGATGGCCACCAGCAGGGTTA  2855
2856  CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagccccagctt  2915
2916  ctctgcccactcatttctagtccagccgcatttcagcagtgccttctggggtgtcccccc  2975
2976  acaccctgctttggccccagaggcgagggaccagtggaggtgccagggaggccccaggac  3035
3036  cctgtggtcccctggctctgcctccccaccctggggtggggctcccggccacctgtctt  3095
3096  gctcctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg  3155
3156  cctctccattatttatttgctctgctctcaggaagcgacgtgacccctgccccagctgga  3215
3216  acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca  3275
3276  gcctgcgcctgagctgcatgcgccaccattttggcagcgtggcagctttgcaaggggct  3335
3336  ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt  3395
3396  gctcaataaatgtttattcattgaaaaaaaaaaaaaa  3433
```

FIG. 17 CONT'D

FIG. 18

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR3 INCLUDING THE 5'UTR (nt -684 TO nt 0), CODING REGION (nt1 TO 2889) AND 3'UTR (nt 2890 TO nt 3418)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625

-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565

-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505

-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445

-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385

-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca  -325

-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265

-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205

-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145

-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85

 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25

 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35
   1                           M  P  R  V  V  G  P  G  A  N  L  C   12

  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95
  13   F  Q  V  R  E  R  G  S  C  C  S  S  R  L  R  L  A  A  N  H   32

  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155
  33   I  W  E  W  P  P  C  A  P  V  I  T  T  V  A  L  K  Q  L  A   52

 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT  215
  53   A  L  L  L  V  H  V  G  G  G  F  L  E  P  P  P  L  A  G  F   72

 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275
  73   C  L  T  P  L  S  F  P  C  R  L  S  S  A  D  G  P  G  A  G   92

 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGGAGGTGGCTGAGCTCCCCGG  335
  93   M  A  D  S  S  E  G  P  R  A  G  P  G  E  V  A  E  L  P  G   112

 336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
 113   D  E  S  G  T  P  G  G  E  A  F  P  L  S  S  L  A  N  L  F   132

 396  TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC  455
 133   E  G  E  D  G  S  L  S  P  S  P  A  D  A  S  R  P  A  G  P   152

 456  AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC  515
 153   G  D  G  R  P  N  L  R  M  K  F  Q  G  A  F  R  K  G  V  P   172

 516  CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA  575
 173   N  P  I  D  L  L  E  S  T  L  Y  E  S  S  V  V  P  G  P  K   192
```

```
576  GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA  635
193   K  A  P  M  D  S  L  F  D  Y  G  T  Y  R  H  H  S  S  D  N   212

636  CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCAAAGCCCCTGCCCC  695
213   K  R  W  R  K  K  I  I  E  K  Q  P  Q  S  P  K  A  P  A  P   232

696  TCAGCCGCCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG  755
233   Q  P  P  P  I  L  K  V  F  N  R  P  I  L  F  D  I  V  S  R   252

756  GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCCACAAGAAACGCCT  815
253   G  S  T  A  D  L  D  G  L  L  P  F  L  L  T  H  K  K  R  L   272

816  AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT  875
273   T  D  E  E  F  R  E  P  S  T  G  K  T  C  L  P  K  A  L  L   292

876  GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC  935
293   N  L  S  N  G  R  N  D  T  I  P  V  L  L  D  I  A  E  R  T   312

936  CGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC  995
313   G  N  M  R  E  F  I  N  S  P  F  R  D  I  Y  Y  R  G  Q  T   332

996  AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAACTTCTCGTGGCCCA  1055
333   A  L  H  I  A  I  E  R  R  C  K  H  Y  V  E  L  L  V  A  Q   352

1056 GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGGG  1115
353   G  A  D  V  H  A  Q  A  R  G  R  F  F  Q  P  K  D  E  G  G   372

1116 CTACTTCTACTTTGGGGAGCTGCCCCTGTCGCTGGCTGCCTGCACCAACCAGCCCCACAT  1175
373   Y  F  Y  F  G  E  L  P  L  S  L  A  A  C  T  N  Q  P  H  I   392

1176 TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG  1235
393   V  N  Y  L  T  E  N  P  H  K  K  A  D  M  R  R  Q  D  S  R   412

1236 AGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA  1295
413   G  N  T  V  L  H  A  L  V  A  I  A  D  N  T  R  E  N  T  K   432

1296 GTTTGTTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCCTCTTCCCCGACAG  1355
433   F  V  T  K  M  Y  D  L  L  L  L  K  C  A  R  L  F  P  D  S   452

1356 CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGATGGCTGCCAAGAC  1415
453   N  L  E  A  V  L  N  N  D  G  L  S  P  L  M  M  A  A  K  T   472

1416 GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG  1475
473   G  K  I  G  I  F  Q  H  I  I  R  R  E  V  T  D  E  D  T  R   492

1476 GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGTATTCCTCGCTTTATGA  1535
493   H  L  S  R  K  S  K  D  W  A  Y  G  P  V  Y  S  S  L  Y  D   512

1536 CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGATCCTGGTGTACAA  1595
513   L  S  S  L  D  T  C  G  E  E  A  S  V  L  E  I  L  V  Y  N   532

1596 CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG  1655
533   S  K  I  E  N  R  H  E  M  L  A  V  E  P  I  N  E  L  L  R   552

1656 GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG  1715
553   D  K  W  R  K  F  G  A  V  S  F  Y  I  N  V  V  S  Y  L  C   572
```

FIG. 18CONT'D

```
1716  TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA  1775
573    A  M  V  I  F  T  L  T  A  Y  Y  Q  P  L  E  G  T  P  P  Y   592

1776  CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC  1835
593    P  Y  R  T  T  V  D  Y  L  R  L  A  G  E  V  I  T  L  F  T   612

1836  TGGGGTCCTGTTCTTCTTCACCAACATCAAAGACTTGTTCATGAAGAAATGCCCTGGAGT  1895
613    G  V  L  F  F  F  T  N  I  K  D  L  F  M  K  K  C  P  G  V   632

1896  GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCCTGGT  1955
633    N  S  L  F  I  D  G  S  F  Q  L  L  Y  F  I  Y  S  V  L  V   652

1956  GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT  2015
653    I  V  S  A  A  L  Y  L  A  G  I  E  A  Y  L  A  M  M  V  F   672

2016  TGCCCTGGTCCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG  2075
673    A  L  V  L  G  W  M  N  A  L  Y  F  T  R  G  L  K  L  T  G   692

2076  GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCCTGCTCGT  2135
693    T  Y  S  I  M  I  Q  K  I  L  F  K  D  L  F  R  F  L  L  V   712

2136  CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC  2195
713    Y  L  L  F  M  I  G  Y  A  S  A  L  V  S  L  L  N  P  C  A   732

2196  CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG  2255
733    N  M  K  V  C  N  E  D  Q  T  N  C  T  V  P  T  Y  P  S  C   752

2256  CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT  2315
753    R  D  S  E  T  F  S  T  F  L  L  D  L  F  K  L  T  I  G  M   772

2316  GGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGGTCTTCATCATCCTGCTGGT  2375
773    G  D  L  E  M  L  S  S  T  K  Y  P  V  V  F  I  I  L  L  V   792

2376  GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA  2435
793    T  Y  I  I  L  T  S  V  L  L  L  N  M  L  I  A  L  M  G  E   812

2436  GACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGAAGCTGCAGTGGGCCACCAC  2495
813    T  V  G  Q  V  S  K  E  S  K  H  I  W  K  L  Q  W  A  T  T   832

2496  CATCCTGGACATTGAGCGCTCCTTCCCCGTATTCCTGAGGAAGGCCTTCCGCTCTGGGGA  2555
833    I  L  D  I  E  R  S  F  P  V  F  L  R  K  A  F  R  S  G  E   852

2556  GATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACCGCAGGTGGTGCTTCAGGGT  2615
853    M  V  T  V  G  K  S  S  D  G  T  P  D  R  R  W  C  F  R  V   872

2616  GGATGAGGTGAACTGGTCTCACTGGAACCAGAACTTGGGCATCATCAACGAGGACCCGGG  2675
873    D  E  V  N  W  S  H  W  N  Q  N  L  G  I  I  N  E  D  P  G   892

2676  CAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCGTGGGCCGCCTCCGCAGGGA  2735
893    K  N  E  T  Y  Q  Y  Y  G  F  S  H  T  V  G  R  L  R  R  D   912

2736  TCGCTGGTCCTCGGTGGTACCCCGCGTGGTGGAACTGAACAAGAACTCGAACCCGGACGA  2795
913    R  W  S  S  V  V  P  R  V  V  E  L  N  K  N  S  N  P  D  E   932

2796  GGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCCGCTGCGATGGCCACCAGCAGGGTTA  2855
933    V  V  V  P  L  D  S  M  G  N  P  R  C  D  G  H  Q  Q  G  Y   952
```

FIG. 18CONT'D

2856 CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagccccagctt 2915
953 P R K W R T D D A P L 963

2916 ctctgcccactcatttctagtccagccgcatttcagcagtgccttctggggtgtcccccc 2975

2976 acaccctgctttggccccagaggcgagggaccagtggaggtgccagggaggccccaggac 3035

3036 cctgtggtcccctggctctgcctccccaccctggggtgggggctcccggccacctgtctt 3095

3096 gctcctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg 3155

3156 cctctccattatttatttgctctgctctcaggaagcgacgtgacccctgccccagctgga 3215

3216 acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca 3275

3276 gcctgcgcctgagctgcatgcgccaccatttttggcagcgtggcagctttgcaaggggct 3335

3336 ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt 3395

3396 gctcaataaatgtttattcattgaaaaaaaaaaaaaa 3433

FIG. 18CONT'D

FIG. 19

AMINO ACID SEQUENCE OF hVR3

```
1    MPRVVGPGAN LCFQVRERGS CCSSRLRLAA NHIWEWPPCA PVITTVALKQ
51   LAALLLVHVG GGFLEPPPLA GFCLTPLSFP CRLSSADGPG AGMADSSEGP
101  RAGPGEVAEL PGDESGTPGG EAFPLSSLAN LFEGEDGSLS PSPADASRPA
151  GPGDGRPNLR MKFQGAFRKG VPNPIDLLES TLYESSVVPG PKKAPMDSLF
201  DYGTYRHHSS DNKRWRKKII EKQPQSPKAP APQPPPILKV FNRPILFDIV
251  SRGSTADLDG LLPFLLTHKK RLTDEEFREP STGKTCLPKA LLNLSNGRND
301  TIPVLLDIAE RTGNMREFIN SPFRDIYYRG QTALHIAIER RCKHYVELLV
351  AQGADVHAQA RGRFFQPKDE GGYFYFGELP LSLAACTNQP HIVNYLTENP
401  HKKADMRRQD SRGNTVLHAL VAIADNTREN TKFVTKMYDL LLLKCARLFP
451  DSNLEAVLNN DGLSPLMMAA KTGKIGIFQH IIRREVTDED TRHLSRKSKD
501  WAYGPVYSSL YDLSSLDTCG EEASVLEILV YNSKIENRHE MLAVEPINEL
551  LRDKWRKFGA VSEYINVVSY LCAMVIETLT AYYQPLEGTP PYPYRTTVDY
601  LRLAGEVITL FTGVLFFFTN IKDLFMKKCP GVNSLFIDGS FQLLYFIYSV
651  LVIVSAALYL AGIEAYLAMM VFALVLGWMN ALYFTRGLKL TGTYSIMIQK
701  ILFKDLFRFL LVYLLFMIGY ASALVSLLNP CANMKVCNED QTNCTVPTYP
751  SCRDSETFST FLLDLFKLTI GMGDLEMLSS TKYPVVFIIL LVTYIILTFV
801  LLLNMLIALM GETVGQVSKE SKHIWKLQWA TTILDIERSF PVFLRKAFRS
851  GEMVTVGKSS DGTPDRRWCF RVDEVNWSHW NQNLGIINED PGKNETYQYY
901  GFSHTVGRLR RDRWSSVVPR VVELNKNSNP DEVVVPLDSM GNPRCDGHQQ
951  GYPRKWRTDD APL
```

Key

Transmembrane domains

Ankyrin binding domains

FIG. 20

FULL-LENGTH hVR3 CLONED INTO (A) pBLUESCRIPT SK(+) (hVR3pBSK) AND (B) pCDNA3.1(+) (hVR1pCDNA3.1) VIA NotI/XhoI RESTRICTION SITES.

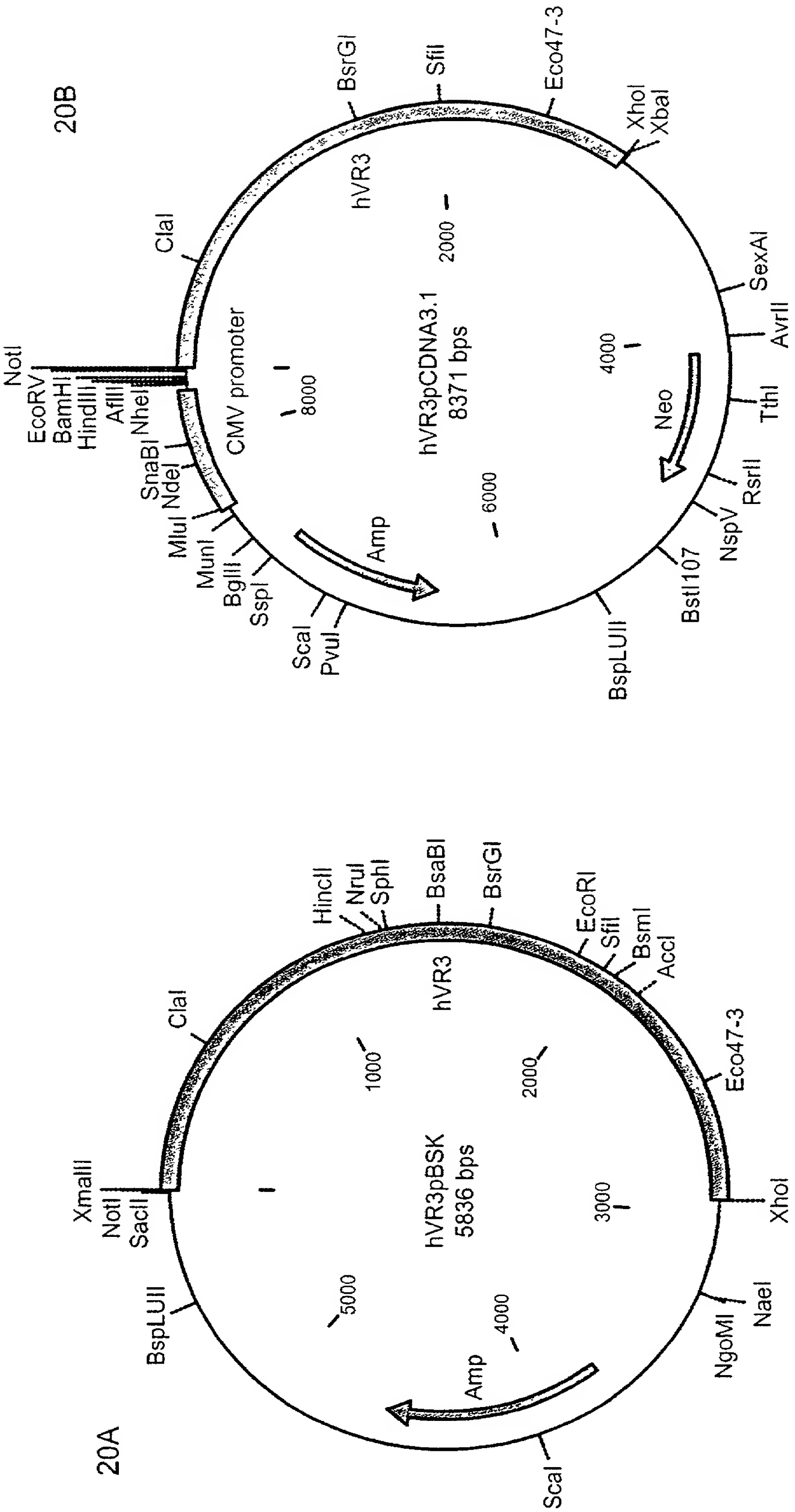


FIG. 21

A MULTIPLE COMPARISON OF THE AMINO ACID SEQUENCES OF THE RAT VR1 AND THE HUMAN VANILLOID RECEPTORS, hVR1, hVRL-1 AND hRV3

```
                     10        20        30        40        50
VR1      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVRL-1   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3     MPRVVGPGANLCFQVRERGSCCSSRLRLAANHIWEWPPCAPVITTVALKQ

                     60        70        80        90       100
VR1      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVRL-1   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3     LAALLLVHVGGGFLEPPPLAGFCLTPLSFPCRLSSADGPGAGMADSSEGP

                    110       120       130       140       150
VR1      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MEQRASLDSEESESPPQENSCL
hVR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MKKWSSTDLGAAADPLQKDTCP
hVRL-1   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3     RAGPGEVAELPGDESGTPGGEAFPLSSLANLFEGEDGSLSPSPADASRPA

                    160       170       180       190       200
VR1      DPPDRDPNCKPPPVKPHIFTTRSRTRLFG....KGDSEEASPLDCPYEEG
hVR1     DPLDGDPNSRPPPAKPQLSTAKSRTRLFG....KGDSEEAFPVDCPHEEG
hVRL-1   ~~~~~~~~~~~~~~~~~~MTSPSSSPVFRLETLDGGQEDGSEADRGKLDF
hVR3     GPGDGRPNLRMKFQGAFRKGVPNP.....IDLLESTLYESSVVPGPKKAP

                    210       220       230       240       250
VR1      GLASCPIITVSSVLTIQRPGDGPASVRPSSQDSVSAG.EKP.PRLYDRRS
hVR1     ELDSCPTITVSPVITIQRPGDGPTGARLLSQDSVAASTEKT.LRLYDRRS
hVRL-1   GSGLPPM..ESQFQGEDRKFAPQIRVNLNYRKGTGASQPDP.NR.FDRDR
hVR3     MDSLFDYGTYRHHSSDNKRWRKKIIEKQPQSPKAPAPQPPPILKVFNRPI

                    260       270       280       290       300
VR1      IFDAVAQSNCQELESLLPFLQRSKKRLTDSEFKDPETGKTCLLKAMLNLH
hVR1     IFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFKDPETGKTCLLKAMLNLH
hVRL-1   LFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCLMKAVLNLK
hVR3     LFDIVSRGSTADLDGLLPFLLTHKKRLTDEEFREPSTGKTCLPKALLNLS

                    310       320       330       340       350
VR1      NGQNDTIALLLDVARKTDSLKQFVNASYTDSYYKGQTALHIAIERRNMTL
hVR1     DGQNTTIPLLLEIARQTDSLKELVNASYTDSYYKGQTALHIAIERRNMAL
hVRL-1   DGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQC
hVR3     NGRNDTIPVLLDIAERTGNMREFINSPFRDIYYRGQTALHIAIERRCKHY

                    360       370       380       390       400
VR1      VTLLVENGADVQAAANGDFFKKTKGRPGFYFGELPLSLAACTNQLAIVKF
hVR1     VTLLVENGADVQAAAHGDFFKKTKGRPGFYFGELPLSLAACTNQLGIVKF
hVRL-1   VKLLVENGANVHARACGRFFQKGQG.TCFYFGELPLSLAACTKQWDVVSY
hVR3     VELLVAQGADVHAQARGRFFQPKDEGGYFYFGELPLSLAACTNQPHIVNY

                    410       420       430       440       450
VR1      LLQNSWQPADISARDSVGNTVLHALVEVADNTVDNTKFVTSMYNEILILG
hVR1     LLQNSWQTADISARDSVGNTVLHALVEVADNTADNTKFVTSMYNEILILG
hVRL-1   LLENPHQPASLQATDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAG
hVR3     LTENPHKKADMRRQDSRGNTVLHALVAIADNTRENTKFVTKMYDLLLLKC

                    460       470       480       490       500
VR1      AKLHPTLKLEEITNRKGLTPLALAASSGKIGVLAYILQREIHEPECRHLS
hVR1     AKLHPTLKLEELTNKKGMTPLALAAGTGKIGVLAYILQREIQEPECRHLS
hVRL-1   ARLCPTVQLEDIRNLQDLTPLKLAAKEGKIEIFRHILQREFS..GLSHLS
hVR3     ARLFPDSNLEAVLNNDGLSPLMMAAKTGKIGIFQHIIRREVTDEDTRHLS
```

```
                     510        520        530        540        550
VR1        RKFTEWAYGPVHSSLYDLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVR1       RKFTEWAYGPVHSSLYDLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVRL-1     RKFTEWCYGPVRVSLYDLASVDSC.EENSVLEIIAF.HCKSPHRHRMVVL
hVR3       RKSKDWAYGPVYSSLYDLSSLDTCGEEASVLEILVY.WSKIENRHEMLAV

                     560        570        580        590        600
VR1        EPLNRLLQDKWDRFVKRIFYFNFFVYCLYMIIFTAAAYYRPV..EGLPPY
hVR1       EPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIFTMAAYYRPV..DGLPPF
hVRL-1     EPLNKLLQAKWDLLIPK.FFLNFLCNLIYMFIFTAVAYHQPTLKKQAAPH
hVR3       EPINELLRDKWRKFGAVSFYINVVSYLCAMVIFTLTAYYQPL..EGTPPY

                     610        620        630        640        650
VR1        KLKNTVGDYFRVTGEILSVSGGVYFFFRGIQ.YFLQRRPSLKSLFVDSYS
hVR1       KMEN.IGDYFRVTGEILSVLGGVYFFFRGIQ.YFLQRRPSMKTLFVDSYS
hVRL-1     .LNAEVGNSMLLTGHILILLGGIYLLVGQLW.YFWRRHVFIWISFIDSYF
hVR3       PYRTTV.DYLRLAGEVITLFTGVLFFFTNIKDLFMKKCPGVNSLFIDGSF

                     660        670        680        690        700
VR1        EILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGWTNMLYYTRGFQQM
hVR1       EMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGWTNMLYYTRGFQQM
hVRL-1     EILFLFQALLTVVSQVLCFLAIEWYLPLLVSALVLGWLNLLYYTRGFQHT
hVR3       QLLYFIYSVLVIVSAALYLAGIEAYLAMMVFALVLGWMNALYFTRGLKLT

                     710        720        730        740        750
VR1        GIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLIEDGKNNSLP....
hVR1       GIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLIEDGKNDSLP....
hVRL-1     GIYSVMIQKVILRDLLRFLLIYLVFLFGFAVALVSLSQEAWRPEAPTGPN
hVR3       GTYSIMIQKILFKDLFRFLLVYLLFMIGYASALVSLLNPCANMKVCNEDQ

                     760        770        780        790        800
VR1        MESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLEFTENYDFKA
hVR1       SESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLEFTENYDFKA
hVRL-1     ATESVQPMEGQEDEGNGAQYRGILEASLELFKFTIGMGELAFQEQLHFRG
hVR3       TNCTVPTY..PSCR.DSETFSTFL...LDLFKLTIGMGDLEMLSSTKYPV

                     810        820        830        840        850
VR1        VFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWKLQRAITILD
hVR1       VFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWKLQRAITILD
hVRL-1     MVLLLLLAYVLLTYILLLNMLIALMSETVNSVATDSWSIWKLQKAISVLE
hVR3       VFIILLVTYIILTSVLLLNMLIALMGETVGQVSKESKHIWKLQWATTILD

                     860        870        880        890        900
VR1        TEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVNWTTWNTNVG
hVR1       TEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVNWTTWNTNVG
hVRL-1     MENGYWWC.RKKQRAGVMLTVGTKPDGSPDERWCFRVEEVNWASWEQTLP
hVR3       IERSFPVFLRKAFRSGEMVTVGKSSDGTPDRRWCFRVDEVNWSHWNQNLG

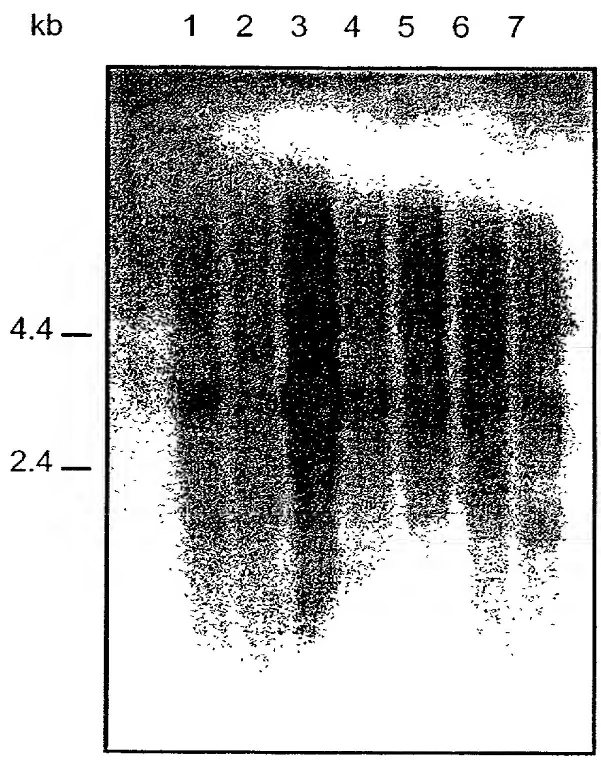
                     910        920        930        940        950
VR1        IINEDPGNCE.....GVKRTLSFSLRSG.........RVSGRNWKNFALV
hVR1       IINEDPGNCE.....GVKRTLSFSLRSS.........RVSGRHWKNFALV
hVRL-1     TLCEDPSGA......GVPRTLENPVLAS.....PPKEDEDGASEENYVPV
hVR3       IINEDPGKWETYQYYGFSHTVGRLRRDRWSSVVPRVVELNKNSNPDEVVV

                     960        970        980        990
VR1        PLLRDASTRDRHATQQEEVQLKHYTGSLKPEDAEVFKDSMVPGEN
hVR1       PLLREASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEN
hVRL-1     QLLQSN~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3       PLDSMGNPRCDGHQQGYPRKWRTDDAPL~~~~~~~~~~~~~~~~~
```

FIG. 21 CONT'D

FIG. 22A

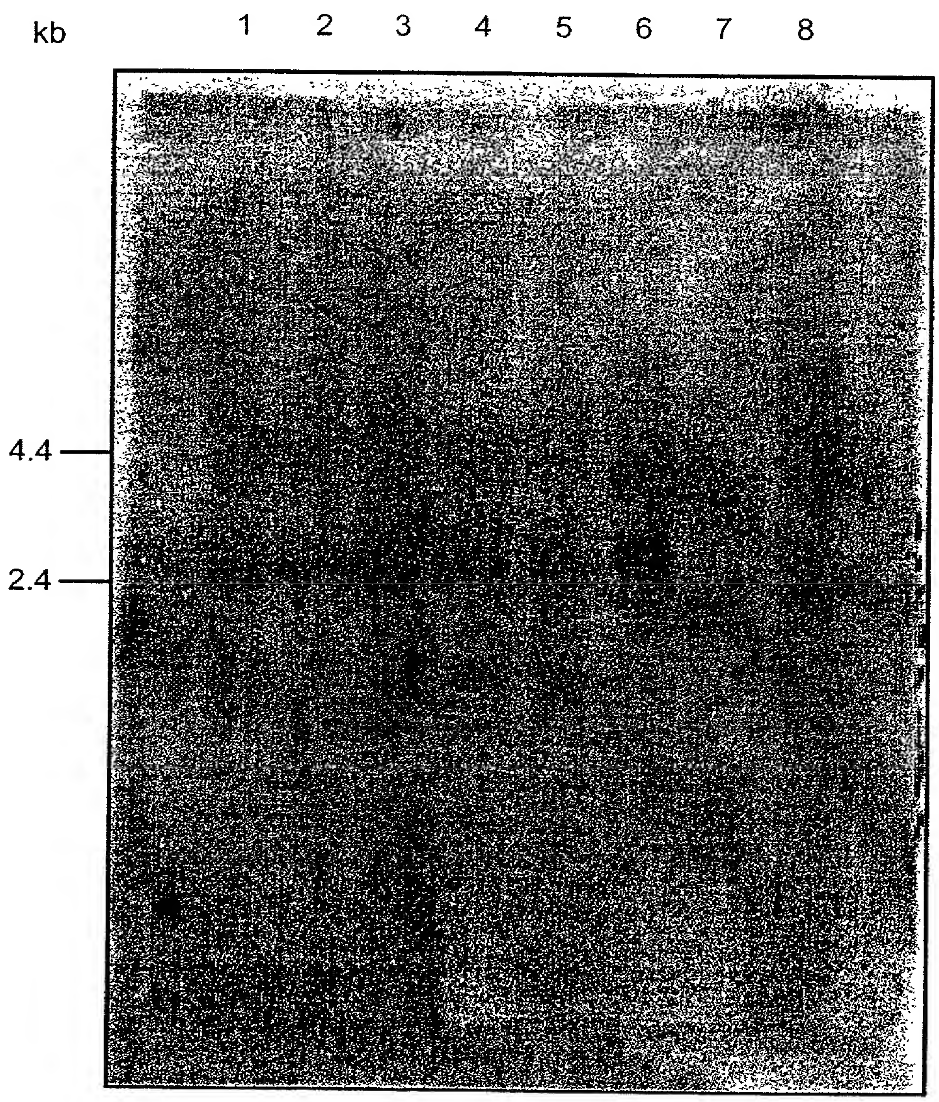
HYBRIDISATION OF A NORTHERN BLOT WITH hVR3



LANE 1: BONE MARROW
LANE 2: ADRENAL GLAND
LANE 3: TRACHEA
LANE 4: LYMPH NODE
LANE 5: SPINAL CORD
LANE 6: THYROID
LANE 7: STOMACH

FIG. 22B

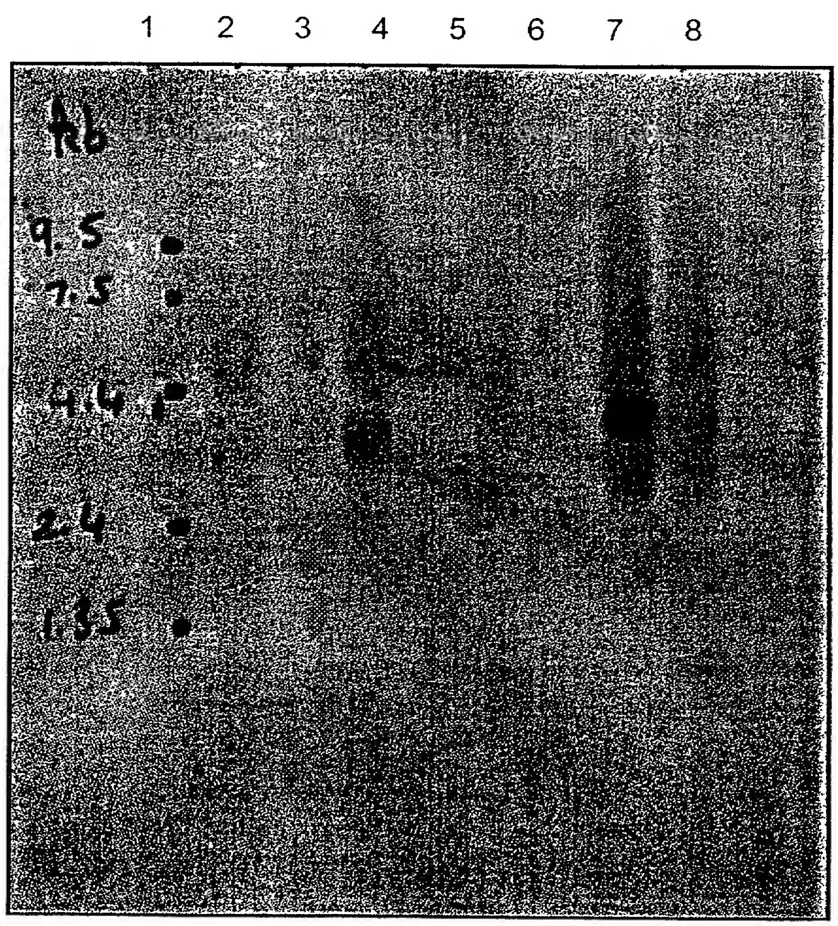
HYBRIDISATION OF NORTHERN BLOT WITH hVR3 PROBE



LANE 1: PERIPHERAL BLOOD LEUKOCYTE
LANE 2: COLON
LANE 3: SMALL INTESTINE
LANE 4: UTERUS
LANE 5: TESTIS
LANE 6: PROSTATE
LANE 7: THYROID
LANE 8: SPLEEN

FIG. 22C

HYBRIDISATION OF A MULTI-TISSUE NORTHERN BLOT WITH THE hVR3 PROBE



LANE 1: HEART
LANE 2: BRAIN
LANE 3: PLACENTA
LANE 4: LUNG
LANE 5: LIVER
LANE 6: SKELETAL MUSCLE
LANE 7: KIDNEY
LANE 8: PANCREAS

SEQUENCE LISTING

<110> Glaxo Group Ltd
Tate, Simon N
Delany, Natalie S
Sanseau, P

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tgtataagct cagtggctgt ggcagcgagg ttgaagagca aaggcaggcc gggcacctgg 180

ctgatgatgt gtggacccgt tgcacagcag ggcccgcagt gcggtgtggg tgtgggtggg 240

ccagtctctg ccgctcaccc tattccaggg acacagtctg cttggctctt ctggactgag 300

ccatcctcat caccgagatc ctccctgaat tcagcccacg acagccaccc cggccgtttt 360

ccttgttctg tgtgggaagg gaggcagcgc ggtggttatc aacctcaccc tgcagaggag 420

gcacctgagg cccagagacg aggagggatg ggtctaaccc agaaccacag atggctctga 480

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gctaggcctg ctcacctctg aggcctctgg ggtgagaggt tcagtcctgg aaacacttca 600

gttctagggg gctgggggca gcagcaagtt ggagttttgg ggtaccctgc ttcacagggc 660

ccttggcaag gagggcaggt ggggtctaag gacaagcagt ccttactttg ggagtcaacc 720

ccggcgtggt ggctgctgca ggttgcacac tgggccacag aggatccagc aagg atg 777
Met
1

aag aaa tgg agc agc aca gac ttg ggg gca gct gcg gac cca ctc caa 825
Lys Lys Trp Ser Ser Thr Asp Leu Gly Ala Ala Ala Asp Pro Leu Gln
5 10 15

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Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg Pro
20 25 30

cct cca gcc aag ccc cag ctc tcc acg gcc aag agc cgc acc cgg ctc 921
Pro Pro Ala Lys Pro Gln Leu Ser Thr Ala Lys Ser Arg Thr Arg Leu
35 40 45

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50 55 60 65

gag gaa ggt gag ctg gac tcc tgc ccg acc atc aca gtc agc cct gtt 1017
Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro Val
70 75 80

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Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu Leu
85 90 95

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Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu Thr
130 135 140 145

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gcc atg ctc aac ctg cac gac gga cag aac acc acc atc ccc ctg ctc 1305
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165 170 175

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180 185 190

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Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile Ala
195 200 205

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Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr Lys
230 235 240

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Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala
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tgc acc aac cag ctg ggc atc gtg aag ttc ctg ctg cag aac tcc tgg 1593
Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser Trp
260 265 270

cag acg gcc gac atc agc gcc agg gac tcg gtg ggc aac acg gtg ctg 1641
Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val Leu
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His Ala Leu Val Glu Val Ala Asp Asn Thr Ala Asp Asn Thr Lys Phe
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gtg acg agc atg tac aat gag att ctg atc ctg ggg gcc aaa ctg cac 1737
Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu His
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ccg acg ctg aag ctg gag gag ctc acc aac aag aag gga atg acg ccg 1785
Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr Pro
325 330 335

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Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr Ile
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Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp Leu
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Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
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ccg ctg aac cga ctc ctg cag gac aag tgg gac aga ttc gtc aag cgc 2073
Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
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Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile Phe
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Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe Lys
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Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu Ser
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Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe Leu
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Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser Glu
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Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser Leu
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Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp Leu
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Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser Thr
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gag tcc acg tcg cac agg tgg cgg ggg cct gcc tgc agg ccc ccc gat 2649
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Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
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Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys
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gct gtc ttc atc atc ctg ctg ctg gcc tat gta att ctc acc tac atc    2793
Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
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ctc ctg ctc aac atg ctc atc gcc ctc atg ggt gag act gtc aac aag    2841
Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn Lys
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atc gca cag gag agc aag aac atc tgg aag ctg cag aga gcc atc acc    2889
Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
690                 695                 700                 705

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Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala Phe
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cgc tca ggc aag ctg ctg cag gtg ggg tac aca cct gat ggc aag gac    2985
Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys Asp
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gac tac cgg tgg tgc ttc agg gtg gac gag gtg aac tgg acc acc tgg    3033
Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr Trp
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Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly
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gtc aag cgc acc ctg agc ttc tcc ctg cgg tca agc aga gtt tca ggc    3129
Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser Gly
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gct cga gat agg cag tct gct cag ccc gag gaa gtt tat ctg cga cag 3225
Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg Gln
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ttt tca ggg tct ctg aag cca gag gac gct gag gtc ttc aag agt cct 3273
Phe Ser Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Ser Pro
820 825 830

gcc gct tcc ggg gag aag tga ggacgtcacg cagacagcac tgtcaacact 3324
Ala Ala Ser Gly Glu Lys
835

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35 40 45

Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
65 70 75 80

Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
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Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
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Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
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Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
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Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
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Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
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Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile
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Ala Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn
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Gly Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr
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Lys Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
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Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser
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Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val
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Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu
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His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr
325 330 335

Pro Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr
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Glu Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys
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Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile
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Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe
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Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
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675 680 685

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Phe Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys
725 730 735

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740 745 750

Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu
755 760 765

Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser
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Gly Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala
785 790 795 800

Ser Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg
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Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Ser Pro Leu Asp Cys Pro
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Tyr Glu Glu Gly Gly Leu Ala Ser Cys Pro Ile Ile Thr Val Ser Ser
65 70 75 80

Val Leu Thr Ile Gln Arg Pro Gly Asp Gly Pro Ala Ser Val Arg Pro
85 90 95

Ser Ser Gln Asp Ser Val Ser Ala Gly Glu Lys Pro Pro Arg Leu Tyr
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Asp Arg Arg Ser Ile Phe Asp Ala Val Ala Gln Ser Asn Cys Gln Glu
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Leu Glu Ser Leu Leu Pro Phe Leu Gln Arg Ser Lys Lys Arg Leu Thr
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Asp Ser Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu Lys
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Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile Ala
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Ile Glu Arg Arg Asn Met Thr Leu Val Thr Leu Leu Val Glu Asn Gly
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Ala Asp Val Gln Ala Ala Ala Asn Gly Asp Phe Phe Lys Lys Thr Lys
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Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala
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Cys Thr Asn Gln Leu Ala Ile Val Lys Phe Leu Leu Gln Asn Ser Trp
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Gln Pro Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val Leu
275 280 285

His Ala Leu Val Glu Val Ala Asp Asn Thr Val Asp Asn Thr Lys Phe
290 295 300

Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu His
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Pro Thr Leu Lys Leu Glu Glu Ile Thr Asn Arg Lys Gly Leu Thr Pro
325 330 335

Leu Ala Leu Ala Ala Ser Ser Gly Lys Ile Gly Val Leu Ala Tyr Ile
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Leu Gln Arg Glu Ile His Glu Pro Glu Cys Arg His Leu Ser Arg Lys
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Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile Ala
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Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
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Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
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Leu Lys Asn Thr Val Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
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Ser Val Ser Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
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Leu Gln Arg Arg Pro Ser Leu Lys Ser Leu Phe Val Asp Ser Tyr Ser
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Glu Ile Leu Phe Phe Val Gln Ser Leu Phe Met Leu Val Ser Val Val
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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
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Leu Cys Arg Phe Met Phe Val Tyr Leu Val Phe Leu Phe Gly Phe Ser
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675 680 685

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Arg Ser Gly Lys Leu Leu Gln Val Gly Phe Thr Pro Asp Gly Lys Asp
725 730 735

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755 760 765

Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Gly Arg Val Ser Gly
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Arg Asn Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Asp Ala Ser
785 790 795 800

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cctgaagtcc accctctttc agcta atg ccc agg gta gtt gga cct ggg gcc 712

Met Pro Arg Val Val Gly Pro Gly Ala

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Leu Arg Leu Ala Ala Asn His Ile Trp Glu Trp Pro Pro Cys Ala Pro
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Val Ile Thr Thr Val Ala Leu Lys Gln Leu Ala Ala Leu Leu Leu Val
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cac gtg gga ggg ggc ttc ctg gag ccc ccg ccc ctg gcc ggg ttc tgc    904
His Val Gly Gly Gly Phe Leu Glu Pro Pro Pro Leu Ala Gly Phe Cys
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Leu Thr Pro Leu Ser Phe Pro Cys Arg Leu Ser Ser Ala Asp Gly Pro
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Glu Val Ala Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly Glu
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gct ttt cct ctc tcc tcc ctg gcc aat ctg ttt gag ggg gag gat ggc   1096
Ala Phe Pro Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp Gly
            125                 130                 135

tcc ctt tcg ccc tca ccg gct gat gcc agt cgc cct gct ggc cca ggc   1144
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Gly Val Pro Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser
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tcg gtg gtg cct ggg ccc aag aaa gca ccc atg gac tca ctg ttt gac    1288
Ser Val Val Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp
                190                 195                 200

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Tyr Gly Thr Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys
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Lys Ile Ile Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln
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ccg ccc ccc atc ctc aaa gtc ttc aac cgg cct atc ctc ttt gac atc    1432
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Val Ser Arg Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe Leu
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ctg acc cac aag aaa cgc cta act gat gag gag ttt cga gag cca tct    1528
Leu Thr His Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser
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acg ggg aag acc tgc ctg ccc aag gcc ttg ctg aac ctg agc aat ggc    1576
Thr Gly Lys Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly
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Asn Met Arg Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg
315 320 325

ggt cag aca gcc ctg cac atc gcc att gag cgt cgc tgc aaa cac tac 1720
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Val Glu Leu Leu Val Ala Gln Gly Ala Asp Val His Ala Gln Ala Arg
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ggg cgc ttc ttc cag ccc aag gat gag ggg ggc tac ttc tac ttt ggg 1816
Gly Arg Phe Phe Gln Pro Lys Asp Glu Gly Gly Tyr Phe Tyr Phe Gly
365 370 375

gag ctg ccc ctg tcg ctg gct gcc tgc acc aac cag ccc cac att gtc 1864
Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val
380 385 390

aac tac ctg acg gag aac ccc cac aag aag gcg gac atg cgg cgc cag 1912
Asn Tyr Leu Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Arg Gln
395 400 405

gac tcg cga ggc aac aca gtg ctg cat gcg ctg gtg gcc att gct gac 1960
Asp Ser Arg Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp
410 415 420 425

aac acc cgt gag aac acc aag ttt gtt acc aag atg tac gac ctg ctg 2008
Asn Thr Arg Glu Asn Thr Lys Phe Val Thr Lys Met Tyr Asp Leu Leu
430 435 440

ctg ctc aag tgt gcc cgc ctc ttc ccc gac agc aac ctg gag gcc gtg 2056
Leu Leu Lys Cys Ala Arg Leu Phe Pro Asp Ser Asn Leu Glu Ala Val
445 450 455

ctc aac aac gac ggc ctc tcg ccc ctc atg atg gct gcc aag acg ggc 2104
Leu Asn Asn Asp Gly Leu Ser Pro Leu Met Met Ala Ala Lys Thr Gly
460 465 470

aag att ggg atc ttt cag cac atc atc cgg cgg gag gtg acg gat gag 2152
Lys Ile Gly Ile Phe Gln His Ile Ile Arg Arg Glu Val Thr Asp Glu
475 480 485

gac aca cgg cac ctg tcc cgc aag tcc aag gac tgg gcc tat ggg cca 2200
Asp Thr Arg His Leu Ser Arg Lys Ser Lys Asp Trp Ala Tyr Gly Pro
490 495 500 505

gtg tat tcc tcg ctt tat gac ctc tcc tcc ctg gac acg tgt ggg gaa 2248
Val Tyr Ser Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Glu
510 515 520

gag gcc tcc gtg ctg gag atc ctg gtg tac aac agc aag att gag aac 2296
Glu Ala Ser Val Leu Glu Ile Leu Val Tyr Asn Ser Lys Ile Glu Asn
525 530 535

cgc cac gag atg ctg gct gtg gag ccc atc aat gaa ctg ctg cgg gac 2344
Arg His Glu Met Leu Ala Val Glu Pro Ile Asn Glu Leu Leu Arg Asp
540 545 550

aag tgg cgg aag ttc ggg gcc gtc tcc ttc tac atc aac gtg gtc tcc 2392
Lys Trp Arg Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser
555 560 565

tac ctg tgt gcc atg gtt atc ttc act ctc acc gcc tac tac cag ccg 2440
Tyr Leu Cys Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Gln Pro
570 575 580 585

ctg gag ggc aca ccg ccg tac cct tac cgc acc acg gtg gac tac ctg 2488
Leu Glu Gly Thr Pro Pro Tyr Pro Tyr Arg Thr Thr Val Asp Tyr Leu
590 595 600

cgg ctg gct ggc gag gtc att acg ctc ttc act ggg gtc ctg ttc ttc 2536
Arg Leu Ala Gly Glu Val Ile Thr Leu Phe Thr Gly Val Leu Phe Phe
605 610 615

ttc acc aac atc aaa gac ttg ttc atg aag aaa tgc cct gga gtg aat 2584
Phe Thr Asn Ile Lys Asp Leu Phe Met Lys Lys Cys Pro Gly Val Asn
620 625 630

tct ctc ttc att gat ggc tcc ttc cag ctg ctc tac ttc atc tac tct 2632
Ser Leu Phe Ile Asp Gly Ser Phe Gln Leu Leu Tyr Phe Ile Tyr Ser
635 640 645

gtc ctg gtg atc gtc tca gca gcc ctc tac ctg gca ggg atc gag gcc 2680
Val Leu Val Ile Val Ser Ala Ala Leu Tyr Leu Ala Gly Ile Glu Ala
650 655 660 665

tac ctg gcc atg atg gtc ttt gcc ctg gtc ctg ggc tgg atg aat gcc 2728
Tyr Leu Ala Met Met Val Phe Ala Leu Val Leu Gly Trp Met Asn Ala
670 675 680

ctt tac ttc acc cgt ggg ctg aag ctg acg ggg acc tat agc atc atg 2776
Leu Tyr Phe Thr Arg Gly Leu Lys Leu Thr Gly Thr Tyr Ser Ile Met
685 690 695

atc cag aag att ctc ttc aag gac ctt ttc cga ttc ctg ctc gtc tac 2824
Ile Gln Lys Ile Leu Phe Lys Asp Leu Phe Arg Phe Leu Leu Val Tyr
700 705 710

ttg ctc ttc atg atc ggc tac gct tca gcc ctg gtc tcc ctc ctg aac 2872
Leu Leu Phe Met Ile Gly Tyr Ala Ser Ala Leu Val Ser Leu Leu Asn
715 720 725

ccg tgt gcc aac atg aag gtg tgc aat gag gac cag acc aac tgc aca 2920
Pro Cys Ala Asn Met Lys Val Cys Asn Glu Asp Gln Thr Asn Cys Thr
730 735 740 745

gtg ccc act tac ccc tcg tgc cgt gac agc gag acc ttc agc acc ttc 2968
Val Pro Thr Tyr Pro Ser Cys Arg Asp Ser Glu Thr Phe Ser Thr Phe
750 755 760

ctc ctg gac ctg ttt aag ctg acc atc ggc atg ggc gac ctg gag atg 3016
Leu Leu Asp Leu Phe Lys Leu Thr Ile Gly Met Gly Asp Leu Glu Met
765 770 775

ctg agc agc acc aag tac ccc gtg gtc ttc atc atc ctg ctg gtg acc 3064
Leu Ser Ser Thr Lys Tyr Pro Val Val Phe Ile Ile Leu Leu Val Thr
780 785 790

tac atc atc ctc acc tct gtg ctg ctc ctc aac atg ctc att gcc ctc 3112
Tyr Ile Ile Leu Thr Ser Val Leu Leu Leu Asn Met Leu Ile Ala Leu
795 800 805

atg ggc gag aca gtg ggc cag gtc tcc aag gag agc aag cac atc tgg 3160
Met Gly Glu Thr Val Gly Gln Val Ser Lys Glu Ser Lys His Ile Trp
810 815 820 825

aag ctg cag tgg gcc acc acc atc ctg gac att gag cgc tcc ttc ccc 3208
Lys Leu Gln Trp Ala Thr Thr Ile Leu Asp Ile Glu Arg Ser Phe Pro
830 835 840

gta ttc ctg agg aag gcc ttc cgc tct ggg gag atg gtc acc gtg ggc 3256
Val Phe Leu Arg Lys Ala Phe Arg Ser Gly Glu Met Val Thr Val Gly
845 850 855

aag agc tcg gac ggc act cct gac cgc agg tgg tgc ttc agg gtg gat 3304
Lys Ser Ser Asp Gly Thr Pro Asp Arg Arg Trp Cys Phe Arg Val Asp
860 865 870

gag gtg aac tgg tct cac tgg aac cag aac ttg ggc atc atc aac gag 3352
Glu Val Asn Trp Ser His Trp Asn Gln Asn Leu Gly Ile Ile Asn Glu
875 880 885

gac ccg ggc aag aat gag acc tac cag tat tat ggc ttc tcg cat acc 3400
Asp Pro Gly Lys Asn Glu Thr Tyr Gln Tyr Tyr Gly Phe Ser His Thr
890 895 900 905

gtg ggc cgc ctc cgc agg gat cgc tgg tcc tcg gtg gta ccc cgc gtg 3448
Val Gly Arg Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val
910 915 920

gtg gaa ctg aac aag aac tcg aac ccg gac gag gtg gtg gtg cct ctg 3496
Val Glu Leu Asn Lys Asn Ser Asn Pro Asp Glu Val Val Val Pro Leu
925 930 935

gac agc atg ggg aac ccc cgc tgc gat ggc cac cag cag ggt tac ccc 3544
Asp Ser Met Gly Asn Pro Arg Cys Asp Gly His Gln Gln Gly Tyr Pro
940 945 950

cgc aag tgg agg act gat gac gcc ccg ctc tag ggactgcagc ccagccccag 3597
Arg Lys Trp Arg Thr Asp Asp Ala Pro Leu
955 960

cttctctgcc cactcatttc tagtccagcc gcatttcagc agtgccttct ggggtgtccc 3657

cccacaccct gctttggccc cagaggcgag ggaccagtgg aggtgccagg gaggccccag 3717

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cttgctccta tggagtcaca taagccaacg ccagagcccc tccacctcag gccccagccc 3837

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<213> Homo sapiens

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20 25 30

Ile Trp Glu Trp Pro Pro Cys Ala Pro Val Ile Thr Thr Val Ala Leu
35 40 45

Lys Gln Leu Ala Ala Leu Leu Leu Val His Val Gly Gly Gly Phe Leu
50 55 60

Glu Pro Pro Pro Leu Ala Gly Phe Cys Leu Thr Pro Leu Ser Phe Pro
65 70 75 80

Cys Arg Leu Ser Ser Ala Asp Gly Pro Gly Ala Gly Met Ala Asp Ser
85 90 95

Ser Glu Gly Pro Arg Ala Gly Pro Gly Glu Val Ala Glu Leu Pro Gly
100 105 110

Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro Leu Ser Ser Leu
115 120 125

Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser Pro Ser Pro Ala
130 135 140

Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg Pro Asn Leu Arg
145 150 155 160

Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro Asn Pro Ile Asp
165 170 175

Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val Pro Gly Pro Lys
180 185 190

Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr Tyr Arg His His
195 200 205

Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile Glu Lys Gln Pro
210 215 220

Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro Ile Leu Lys Val
225 230 235 240

Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg Gly Ser Thr Ala
245 250 255

Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His Lys Lys Arg Leu
260 265 270

Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys Thr Cys Leu Pro
275 280 285

Lys Ala Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp Thr Ile Pro Val
290 295 300

Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg Glu Phe Ile Asn
305 310 315 320

Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr Ala Leu His Ile
325 330 335

Ala Ile Glu Arg Arg Cys Lys His Tyr Val Glu Leu Leu Val Ala Gln
340 345 350

Gly Ala Asp Val His Ala Gln Ala Arg Gly Arg Phe Phe Gln Pro Lys
355 360 365

Asp Glu Gly Gly Tyr Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
370 375 380

Ala Cys Thr Asn Gln Pro His Ile Val Asn Tyr Leu Thr Glu Asn Pro
385 390 395 400

His Lys Lys Ala Asp Met Arg Arg Gln Asp Ser Arg Gly Asn Thr Val
405 410 415

Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg Glu Asn Thr Lys
420 425 430

Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Leu Lys Cys Ala Arg Leu
435 440 445

Phe Pro Asp Ser Asn Leu Glu Ala Val Leu Asn Asn Asp Gly Leu Ser
450 455 460

Pro Leu Met Met Ala Ala Lys Thr Gly Lys Ile Gly Ile Phe Gln His
465 470 475 480

Ile Ile Arg Arg Glu Val Thr Asp Glu Asp Thr Arg His Leu Ser Arg
485 490 495

Lys Ser Lys Asp Trp Ala Tyr Gly Pro Val Tyr Ser Ser Leu Tyr Asp
500 505 510

Leu Ser Ser Leu Asp Thr Cys Gly Glu Glu Ala Ser Val Leu Glu Ile
515 520 525

Leu Val Tyr Asn Ser Lys Ile Glu Asn Arg His Glu Met Leu Ala Val
530 535 540

Glu Pro Ile Asn Glu Leu Leu Arg Asp Lys Trp Arg Lys Phe Gly Ala
545 550 555 560

Val Ser Phe Tyr Ile Asn Val Val Ser Tyr Leu Cys Ala Met Val Ile
565 570 575

Phe Thr Leu Thr Ala Tyr Tyr Gln Pro Leu Glu Gly Thr Pro Pro Tyr
580 585 590

Pro Tyr Arg Thr Thr Val Asp Tyr Leu Arg Leu Ala Gly Glu Val Ile
595 600 605

Thr Leu Phe Thr Gly Val Leu Phe Phe Phe Thr Asn Ile Lys Asp Leu
610 615 620

Phe Met Lys Lys Cys Pro Gly Val Asn Ser Leu Phe Ile Asp Gly Ser
625 630 635 640

Phe Gln Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val Ile Val Ser Ala
645 650 655

Ala Leu Tyr Leu Ala Gly Ile Glu Ala Tyr Leu Ala Met Met Val Phe
660 665 670

Ala Leu Val Leu Gly Trp Met Asn Ala Leu Tyr Phe Thr Arg Gly Leu
675 680 685

Lys Leu Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys Ile Leu Phe Lys
690 695 700

Asp Leu Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe Met Ile Gly Tyr
705 710 715 720

Ala Ser Ala Leu Val Ser Leu Leu Asn Pro Cys Ala Asn Met Lys Val
725 730 735

Cys Asn Glu Asp Gln Thr Asn Cys Thr Val Pro Thr Tyr Pro Ser Cys
740 745 750

Arg Asp Ser Glu Thr Phe Ser Thr Phe Leu Leu Asp Leu Phe Lys Leu
755 760 765

Thr Ile Gly Met Gly Asp Leu Glu Met Leu Ser Ser Thr Lys Tyr Pro
770 775 780

Val Val Phe Ile Ile Leu Leu Val Thr Tyr Ile Ile Leu Thr Ser Val
785 790 795 800

Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Gly Gln
805 810 815

Val Ser Lys Glu Ser Lys His Ile Trp Lys Leu Gln Trp Ala Thr Thr
820 825 830

Ile Leu Asp Ile Glu Arg Ser Phe Pro Val Phe Leu Arg Lys Ala Phe
835 840 845

Arg Ser Gly Glu Met Val Thr Val Gly Lys Ser Ser Asp Gly Thr Pro
850 855 860

Asp Arg Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Ser His Trp
865 870 875 880

Asn Gln Asn Leu Gly Ile Ile Asn Glu Asp Pro Gly Lys Asn Glu Thr
885 890 895

Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg Leu Arg Arg Asp
900 905 910

Arg Trp Ser Ser Val Val Pro Arg Val Val Glu Leu Asn Lys Asn Ser
915 920 925

Asn Pro Asp Glu Val Val Val Pro Leu Asp Ser Met Gly Asn Pro Arg
930 935 940

Cys Asp Gly His Gln Gln Gly Tyr Pro Arg Lys Trp Arg Thr Asp Asp
945 950 955 960

Ala Pro Leu

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<213> Homo sapiens

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20 25 30

Gly Ser Gly Leu Pro Pro Met Glu Ser Gln Phe Gln Gly Glu Asp Arg
35 40 45

Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr
50 55 60

Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe
65 70 75 80

Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
85 90 95

Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
100 105 110

Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
115 120 125

Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp
130 135 140

Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
145 150 155 160

Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
165 170 175

Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg
180 185 190

Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
195 200 205

Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val
210 215 220

Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
225 230 235 240

Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
245 250 255

Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
260 265 270

Leu Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp
275 280 285

Ile Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu
290 295 300

Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
305 310 315 320

Leu Ser His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val
325 330 335

Arg Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn
340 345 350

Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His
355 360 365

Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp
370 375 380

Asp Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile
385 390 395 400

Tyr Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys
405 410 415

Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
420 425 430

Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val
435 440 445

Gly Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser
450 455 460

Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
465 470 475 480

Thr Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu
485 490 495

Pro Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr
500 505 510

Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln
515 520 525

Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Ile Tyr Leu Val
530 535 540

Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser Gln Glu Ala
545 550 555 560

Trp Arg Pro Glu Ala Pro Thr Gly Pro Asn Ala Thr Glu Ser Val Gln
565 570 575

Pro Met Glu Gly Gln Glu Asp Glu Gly Asn Gly Ala Gln Tyr Arg Gly
580 585 590

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Ile Leu Glu Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly
        595                 600                 605

Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu
    610                 615                 620

Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Ile Leu Leu Leu Asn Met
625                 630                 635                 640

Leu Ile Ala Leu Met Ser Glu Thr Val Asn Ser Val Ala Thr Asp Ser
                645                 650                 655

Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
            660                 665                 670

Asn Gly Tyr Trp Trp Cys Arg Lys Lys Gln Arg Ala Gly Val Met Leu
        675                 680                 685

Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
    690                 695                 700

Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr
705                 710                 715                 720

Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
                725                 730                 735

Pro Val Leu Ala Ser Pro Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu
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Glu Asn Tyr Val Pro Val Gln Leu Leu Gln Ser Asn
        755                 760
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<400> 9
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ctgcagaact cctggcaga 19

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ctggagttag ggtctccatc c 21

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aggcccactc ggtgaacttc 20

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<223> Description of Artificial Sequence: Primer

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<210> 24

<211> 32

<212> DNA

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<223> Description of Artificial Sequence: Primer

<400> 24

tgcactgaat tcgagcactg gtgttccctc ag 32

<210> 25

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Primer

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tgtggacagc tacagtgaga 20

<210> 26

<211> 19

<212> DNA

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<400> 26

gtggaaaacc cgaacaaga 19

<210> 27

<211> 23

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<223> Description of Artificial Sequence: Synthetic sequence

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Asp Ser Glu Glu Ala Ser Cys
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<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

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Cys Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Asp Ser Met
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Val Pro Gly Glu Lys
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<211> 18

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<223> Description of Artificial Sequence: Primer

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cacctcttgt tgtcactgga 20

<210> 33
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<223> Description of Artificial Sequence: Primer

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acaagaaggc ggacatgcgg 20

<210> 40

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<223> Description of Artificial Sequence: Primer

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atctcgtggc ggttctcaat 20

09/857123
JC18 Rec'd PCT/PTO 0 1 JUN 2001

ABSTRACT

HUMAN VANILLOID RECEPTORS AND THEIR USES

The invention provides novel human vanilloid receptor (hVR) proteins, in particular hVR1 and hVR3, nucleotide sequences encoding for the novel hVR proteins, and hVR proteins for use in a method for screening for agents useful in the treatment or prophylaxis of disorders which are responsive to modulation of hVR activity in a human patient. The invention also provides expression vectors comprising said nucleotide sequences, stable cell lines comprising said expression vectors, antibodies specific for the novel hVR proteins, methods for the identification of compounds which exhibit hVR modulating activity, compounds identifiable and identified by such methods, and methods of treatment or prophylaxis of disorders which are responsive to modulation of hVR activity in a human patient.

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY	ATTORNEY'S DOCKET PG3606USW
	First Names Inventor: **DELANY**
( ) Declaration submitted with initial filing or	***Complete if known*:** App No.:
(X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))	Filing Date Concurrently herewith
	Group Art Unit:

23347

PATENT TRADEMARK OFFICE

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

HUMAN VANILLOID RECEPTORS AND THEIR USES

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on ___________as United States application Serial No. _____________ or **PCT** International

Application Number EP99/09284 filed 11/30/1999 and was amended on (MM/DD/YYYY) _________________(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. GB9826359.3	GB	12/01/1998	x
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

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PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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